POPULATION MANAGEMENT OF SPECIES INVOLVED IN HUMAN WILDLIFE CONFLICT

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Wildlife Institute of India

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EXECUTIVE SUMMARY

Reproductive control methods using contraceptives have been described using terms such as “humane” or “benign”, and has steadily gained wide acceptance within the conservation community, managers and policy makers alike. Thus, to investigate the field applicability of reproductive control in conflict mitigation, a project titled “Population Management of Species involved in Human Wildlife Conflict” was awarded to the Wildlife Institute of India (WII) by The Ministry of Environment, Forest and Climate Change, New Delhi, vide its order no. 8 – 98/2016 – WL dated 30th January, 2018. The project aims to develop and implement a range of mitigation strategies including immune-contraception for managing conflict with wild animal populations in the country. For the study, four focal species as identified by the MoEF&CC, Govt of India namely Rhesus macaque (*Macaca mulatta*), Nilgai (*Boselaphus tragocamelus*), Wild pig (*Sus scrofa)* and Elephant (*Elephas maximus*) were selected for the trials at specific sites experiencing severe conflict from above species.

Implementation of immuno-contraception involves interdisciplinary approach. The objectives of current study can only be achieved if it is approached based on scientific principles and in a professional manner in partnerships with research institutes, local communities and various State Forest Departments. Further, reproductive control needs an understanding of optimal demographic age and sex ratios to be maintained for sustenance of species. Hence, it is always practiced only in addition to the ecological population monitoring. Thus, as part of the initial phase, following field sites were selected for ecological monitoring process:

1. **Rhesus macaque :** 5 Km radius of Wildlife Institute of India, Dehradun, and Rajaji Tiger Reserve, Uttarakhand (Permissions secured: Letter from the Office of PCCF (WL) no/ 3125 – 5/6, dated 27th February, 2020).

2. **Nilgai & Wild Pig :** Pench Tiger Reserve, Panna Tiger Reserve, and Chattarpur division, Madhya Pradesh (Permissions secured: Letter from the Office of the Principal Chief Conservator of Forest (Wildlife), Madhya Pradesh vide क्रमांक / व.प्र. /म.चि. / 2752, dated 20/05/2020).

3. **Asiatic Elephants & Wild Pig :** Hassan and Kodagu Districts of Karnataka (Permissions secured: Vide letter no. PCCF(WL)/E(C1)/CR-03/2016 – 17, dated 07/06/2019).

The feasibility of using a particular population control method in target species/population depends on a myriad of parameters including movement pattern, population, sex ratios, age structure, and estimated rate of increase of the target species in study areas.

Rhesus macaque: The *Macaca mulatta* in India, inhabits a wide range of natural and anthropogenic landscapes, living in close association with humans. Increased instances of human-macaque conflict have been reported from Northern India lately. Ecological investigations were made on a representative population of the species in a 16 sq. km study area comprising fragmented forest patches and anthropogenic structures on the outskirts of Dehradun city in Uttarakhand. Population densities were calculated for the area using trail transect distance sampling methods. Overall densities (2020-2022) were observed to be ~97 individuals per sq. km for a combined effort of 325.5 km. High adult ♂: adult ♀ and infant: adult♀ ratios were observed across seasons throughout the duration of the exercise. Ranging patterns in synanthropic macaques are influenced by habitat preference, which may indicate possible conflict scenarios in human-altered habitats. During 2018-2020, one adult ♀ was fitted with a GPS-VHF collar in each of the four socially distinct study troops (A, B, C and D). Home ranges varied from 19.21-39.64 ha. Mean daily travel distances ranged from 1.54±0.06-2.09±04 km. Natural vegetation patches, human habitation and roadsides comprised the maximum land use land cover classes (LULC) of their home ranges. One adult ♀ from each of the study troops A and B were fitted with a GPS-GSM collar in 2022 with data obtained for approximately four months for each. Home ranges varied from 26-47 ha. Overall daily travel distances varied from 1.92±0.04-2.62±0.06 km. Habitat composition showed a similar trend for LULC from 2018-2020. Design III habitat selection ratios were calculated using habitat class areas from 100% minimum convex polygon as available and 95% kernel as utilized. Selection

ratios were high for human-modified habitat classes. Behavioural observations were undertaken using scan and focal sampling methods based on an ethogram for the behavioural states of the species. Troops were observed to show a higher percentage of time feeding on anthropogenic food resources. Small home ranges with high selection ratios for roadsides and human habitation suggest higher resource availability for the troops through provisioning and garbage dumps. High revisits and extended residence at localized sites may perpetuate heightened perceived agonistic interactions in humans. Understanding these patterns shall allow for informed management practices alleviating conflict, facilitating long-term human-macaque coexistence. Field based pregnancy diagnosis by ultrasound examination has also been initiated from December 2020 in order to understand the pregnancy and recruitment rates.

Asian Elephant: To ascertain ecological and biological attributes of Asian elephants, the project team initiated filed work in Kodagu landscape Karnataka in November, 2019. To ascertain the ecological and biological attributes of Asian elephants, the project team-initiated fieldwork in Kodagu landscape Karnataka in November 2019. A total of nine conflict-causing elephant herds (≈98 individuals) were identified and since then are being monitored extensively through radio telemetry and ground tracking to understand the demography, movement ecology, conflict, and reproductive ethology of the species in the landscape. A total of 22 (9 females, 4 resident males, 9 translocated males) elephants have been radio-collared. The home range of resident

females is 70.83 (±10.20) sq.km, with a daily movement of 4.09 km (±0.401), resident males are 39.28 (±15.141) sq.km with a daily movement of 4.59 km (±1.214) and translocated males home range is 221.71 (±174.78) sq.km with a daily movement of 4.51km (±1.42). The majority of resident females and males mostly stay in the coffee plantations rather than in the protected areas which leads to human-elephant conflict instances. Additionally, the socio-economics of the conflict from 1992 - 2020 has also been accessed in the landscape to better understand elephant conflict dynamics. The Western Ghats still faces the highest HEC instances in the state, both central and northern western Ghats have also started exhibiting increasing trends in HEC instances from the data analysed from 2019-2021. Efforts are also being continued to establish a robust genetic capture-mark-recapture-based population estimation technique for the species. Additionally, the project is also assisting the State Forest department in the implementation of a radio-telemetry-based Early Warning System, while simultaneously providing technical support to access translocation as a mitigation measure for the Human-elephant conflict.

Wild pig: Permissions to carry out field activities for assemblage of information on species biology, ecology, and conflict assessment for both the species in free-ranging setup was accorded by Madhya Pradesh Forest Department only by the month of May, 2020 during the pandemic. Camera trapping has been done to analyse the severity of the wild pig conflict in Pench Tiger Reserve (PTR) and 5 km radius area around Wildlife Institute of India (WII) Dehradun. Based on our interactions with farmers, we deployed seven camera traps along the park's western boundary for 5-7 trap nights and 19 camera traps around the Chandrabani in

various villages for 60 trap nights. Preliminary analyses from camera trap photos from PTR revealed frequent visitation by the wild pig, chital, nilgai sambar deer, and black-naped hare. In Dehradun around WII, wild pig, rhesus macaque, jackal, small Indian civet, Asian palm civet, barking deer, sambar black-naped hare, porcupine, and peacock were frequent visitors in wheat fields. Detailed questionnaire surveys with closed and open-ended questions were conducted in various villages around the Wildlife Institute of India to get more insights into the human-wild pig conflict. All farmers reported frustration, especially against wild pigs, as they were hardest to detect at night and caused additional damage by trampling and rooting in both the study sites. We documented current mitigation methods employed in these sample fields from both study sites. Farmers used various methods, including active guarding with or without dogs, barbed wire, mesh wire, brush fencing, lights, reflectors, and cloth/plastic flurry in both the study sites. In Dehradun, the rubble wall has been tried and tested as an initiative of the Uttarakhand Forest Department to reduce human-wild-pig conflict.

Nilgai: Permissions to carry out field activities for assemblage of information on species biology, ecology, and conflict assessment for both the species in free-ranging setup was accorded by Madhya Pradesh Forest Department only by the month of May, 2020 during the pandemic. Camera trapping has been done to analyse the severity of the nilgai conflict in Pench Tiger Reserve (PTR). Camera trap photos from PTR revealed frequent visitation of crop fields by the wild pig, chital, nilgai sambar deer, and black-naped hare. Farmers used various methods, including active guarding with or without dogs, barbed wire, mesh wire, brush fencing, lights, reflectors, and cloth/plastic flurry in both the study sites.

We found that the state forest department do not compensate for crop loss by herbivores and secondary data related crop damage by nilgai were scarce, hence by using Arc-GIS tools, 2 Km buffer clip around the Panna Tiger Reserve boundary was clipped. Villages whose polygons were found within this buffer clip, including those situated at the fringe of core were also selected. A total of 37 villages were chosen alongside the boundary of core and buffer from all sides of Tiger reserve. Semi structured questionnaire survey was conducted with farmers in these villages. Questions were addressed on source of livelihood, extent and frequency of damage, various mitigation measures being adopted by farmers and their perception towards this species. 15-20 farmers from respective households were sampled from each village in random manner. Opportunistic sign survey was also carried out in crop lands to look for the species presence. We surveyed a total of 310 respondents and majority of them were victims suffering crop loss from wild ungulates. Most of the respondent claimed wild pig and nilgai to be the major crop raider species while other ungulate species such as sambar, chital, chinkara and feral cattle was also named frequently by some farmers. Langur and rhesus macaque were claimed as crop raiders of pulses. Although revenue department compensate for crop loss by wild animals, majority of the respondents did not receive any compensation for crop damage. Many of them told that the local revenue officers known as patwari, do not come for scrutiny and process their compensation claim applications. Many of the farmers were unaware of the compensation scheme and procedure too. It was found that compensation was provided based on visual inspection to the area of damage on standing crops, which should be more than 25%

of the total area. Due to the scarcity of irrigation facilities farmers practice agriculture in monsoon and winter seasons only. Farmers were found to be adopting a range of mitigation measures from night guarding and field guarding huts to fencing with thorny shrubs and barbed wire fences. We have found dung pellets and hoof marks of nilgai in the agriculture field. Our review of print media report on human-nilgai conflict have concluded that Bihar state is the worst affected among all other states and have 78 affected tehsils. Uttar Pradesh and Madhya Pradesh states ranked second and third in this list. Vegetable, cereal, and pulse crops were found to be mostly raided by the species. The rate of nilgai-vehicle collisions was estimated to be 13.8 cases per year in the country.

Reproductive biology studies: The efficacy and effectiveness of immuno-contraceptive vaccines vary greatly with age species, age, individual differences in immune-competence, as well as the active component of the vaccine. Further, formulation, dose used, delivery system and frequency of vaccination may also need species specific alterations based on the reproductive characteristics. Therefore, even though the efficacy of commercially available vaccines has been demonstrated in several species over the years, it is imperative to first understand the reproductive biology of the species being targeted before carrying out the trial. In order to develop an advanced 'Wildlife Physiology & Disease Ecology Laboratory' in WII under the project, the project team visited various animal housing and laboratory facilities in 2018 and 2019 including National Institute of Immunology, National Institute of Brain Research, Indian Veterinary Research Institute and Tamil Nadu Veterinary and Animal Sciences University. Based on the visits and expert inputs, a comprehensive building plan was

developed. The laboratory & adjacent veterinary clinic building process has almost been completed and currently undergoing the process of furnishing with essential laboratory equipment. The animal enclosures to carryout vaccine trails are also constructed in WII premises, these enclosures are designed to house non-human primates and small ungulates. On 28th April 2022, Shri Bhupender Yadav, Hon'ble Minister for Environment, Forest & Climate change, Govt. of India inaugurated the “Wildlife Physiology & Disease Ecology Laboratory” at Wildlife Institute of India. The laboratory activities being under taken involve: Sex determination from fecal/dung samples of study species using PCR, identification of genetic markers in study species to assist in genetic capture-mark-recapture, reproductive cyclicity analysis in females of study species via estimation of reproductive hormones in faecal and blood samples, gestational staging using sonography and blood biochemistry from collected blood samples. The health of the targeted population is also closely being monitored through laboratory analysis of collected field samples for presence and prevalence of gastrointestinal parasites and pathogenic *E. coli.* Given that diseases are an important subset of human wildlife conflict, anti-microbial resistance, an emerging global zoonosis was also studied in interface areas.

Since PZP (Porcine Zona Pellucida) based immuno-contraception has been tested successfully in numerous captive and free ranging wildlife species, it was selected as one of the immuno-contraceptive to be studied under the project. Currently, the said vaccines as well as raw materials to produce them are unavailable in India and hence had to be imported. There were however a number of complex technical, and legal issues that need to be addressed before procurement of the same. Since the vaccine is of animal origin and novel to India, import licence, permissions and no objection certificates had to be procured from several regulating agencies. Though there was an ambiguity among the regulating agencies regarding the procedure for importing and field use, it was sorted out by holding repeated consultations with agencies and other stakeholders involved. Subsequently, import license has been obtained from the Directorate General of Health Services, New Delhi, vide letter no X-11026/165/2018-BD dated 12/07/2018. Following this, 200 doses of PZP contraceptive vaccine (100 µg each) were imported from the Science and Conservation Centre, Zoo Montana, USA. Subsequently, all necessary permits for field trials, including No objection certificate (NOC) from Animal Husbandry Ministry, New Delhi; NOC from Directorate General of Health Services, New Delhi; Vaccine safety certificate from National Institute of High Security Animal Diseases, Bhopal were obtained for the vaccines. Additionally, an MoU was signed with the Humane Society International (HSI, India) in 2020, so as to procure additional PZP vaccines from their South African counterparts. Through this MoU, WII is also coordinating with Dr Henk Bertschinger's laboratory at the University of Pretoria, South Africa to procure actual vaccine for initial trials, as well as obtain the technical expertise for future local production.

Immuno-contraception trials were conducted on rats. Immunization of rats for generation of polyclonal and monoclonal antibodies is an indispensable tool in immunological studies. The antibody titer estimated post vaccination can be correlated to the fertility outcome. Also, mating behavior of subject animals and impact on primary and secondary reproductive organs

can be observed to establish the safety of immuno-contraceptive. Among the study species, enough information and field knowledge on species biology and ecology has been collated with reference to elephants and Rhesus Macaques, enabling the project to proceed towards phase 2 of immune-contraception trials in the species. All the necessary permits, with regards to testing of PZP vaccines on Macaques are in place, including the No-Objection Certificate from Drug Control General of India (Letter from the Office of Drug Control General (India), vide File No.X-11026/165/2018 BD, dated 18/02/2019). The trials will be soon initiated on completion of macaque captive facility in WII and acquirement of test subjects. As per the norms, the vaccine trial protocols have been submitted to The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi for approval, vide letter no. No. WII/QQ/HWL – Conflict/2019 – 19/8, Dated 22/06/2021. Due to the writ petition (civil) No.(S) 107/2013 in the Honourable Supreme Court, which withholds the use of population control in Elephants, no progress could be gained in carrying out the PZP efficacy on Asian Elephants in field. The Institute has nonetheless been coordinating with the MoEFCC, Government of India and Humane Society International (India), both of whom are parties in the said petition for vacation of the stay. Subsequent to this, the vaccine trails can be initiated right away, initially on the select captive females of elephant camps and subsequently on the identified conflict herds of Karnataka. An MoU has been signed with Zoo Authority of Karnataka and Sri Chamarajendra Zoological Gardens, Mysuru, Karnataka for initiation of captive trials in Nilgai (Letter from the Office of The Member Secretary, ZAK vide No. MSY/08/ASW/20011 Dated 02-20 19). Permissions for the same has also been obtained from Central Zoo Authority, vide F.No 7-7/2020-CZA(PKR), dated 24/05/2020. However, due to delay in administrative procedures and construction of experiment facility, the said activities were not initiated in time.

Human-wildlife conflicts are multifaceted and cannot be easily explained or resolved. To gain insights into the intensity, complexity, and potential approaches to address these conflicts, we looked into population management scenarios. It is only part of solution other aspects which include socio-political dynamics need separate study. Income levels can also impact human-wildlife conflicts. Communities experiencing poverty may have a higher tolerance for losses caused by wildlife due to their reliance on natural resources for subsistence. A framework for population control is divided into urban and rural conflict area, having gradient of conflict and area which are at interface with Protected Area or Reserve Forest Area where population of species in conflict occupy natural and human habitat. The population modelling was done to assess the outcome of various scenarios for population control of rhesus macaque, elephants, wild pig and nilgai.

Rhesus macaque population control is challenging due to adaptation to human food, behavioural plasticity and cultural protection. The population level scenarios for the natural conditions showed an intrinsic rate of increase (r) for the experimental population to be 8.41%. In a simulated scenario when only 18% of the adult females are allowed to breed, rate of growth declined by 6.4% with an extinction probability of 52% and a population half-life ($t_{1/2}$)of ~11 years. When only 4% of the adult breeding females are allowed to breed, growth rate drops to -17.6% with an extinction probability of one and a $t_{1/2}$ of 4 years. In case of a troop of an average

size of 43, natural conditions show an intrinsic rate of increase at 9.1%. When only 18% of the troop's adult females breed, extinction probability is maximum with growth rate being -6.6% and $t_{1/2}$ of 11 years. When only 4% of the adult breeding females are allowed to breed, growth rate drops to -16.4 and a $t_{1/2}$ of 4 years. To implement this, macaques will be captured and treated for population control. Following scenarios are visualized

a) Rural or urban area

In high conflict areas, capture most of the macaque and move them to rescue centre. All adult monkeys should be sterilised using surgical procedure, the sub adults should be kept separately and their reproductive control should be done at reaching adulthood. In moderate and low conflict area most of the monkeys (100%) should be sterilised using surgical procedure and released in same area. The procedure should be continued for 5 years till entire population is captured and sterilized. Further course of action need to be decided at this juncture for continuation of the program.

b) Rural or urban area with forest interface

In interface area which is either Protected Area or large Reserve forest 100% of adult monkeys should be sterilised using surgical procedure and then the sub adults of that population in following year when they reach adulthood 80% should be sterilised surgically and 10 % adult should be treated with immuno-contraception like PZP for at least 5 years. The population should be monitored and adaptive process should be followed to decide what percentage will be reproductively controlled and the method of contraception surgical or immuno-contraceptive and what proportion in each treatment need to be decided as per situation.

Population and its characteristics need to be regularly monitored using line transect and facial recognition based Mark-Recapture method involving people in affected area.

The whole process need long term hand holding and appropriate training of Veterinary staff of districts to carry out surgical procedure. Manual for surgical procedure is developed to appropriately deal with the process. The problem will not be resolved in doing only sterilization but it involves maintain them for lifetime in captivity ensuring no breeding happens in rescue centre. The low population areas which is reproductively controlled in urban and rural area and maintained at low densities need to be monitored and controlled for long term. This will need resources and long term financial assistance. People need to be engaged to fund for upkeep of macaque in captivity, religious agencies can be roped in to assist in maintaining them in captivity.

Elephant population is expanding its range. They are occupying new areas in Chattisgarh, Madhya Pradesh, Maharashtra and many other places. The isolated population or population close to protected areas are generally more in conflicts. We modelled scenarios for population control on herd basis. The scenarios for the natural conditions in natural condition with 30% breeding females with average herd size of 40 showed an intrinsic rate of increase (r) to be

5.1%. When 15% of the females are breeding, growth rate will be 2.7% and when 3 % of the adult females are allowed to breed, growth rate drops to -2.6% with an extinction probability of 0.96 and population will reduced to half in 26 years. Immuno-contraceptive like PZP which is found safe in African elephants should be used initially for 5 years and then decision should be taken based on local condition to continue the program depending on population monitoring data. Herd need to be monitored physically and using camera trap based distance method to check demographic parameters which will assist in making population management decisions.

The wild pig population control is a big challenge as they grow very fast in ideal condition of food availability. The intrinsic rate of growth rate in natural optimum habitat is 37.7%. When only 10% of the females are allowed to breed, growth rate r drops to -9.9% and population will be reduced to half in 7 years and when adult female breeding population is reduced to 5% growth rate will reduce to -19.5, and population will become half in 4 years. There is possibility of chemical control but these procedures are costly and logistically challenging. It is suggested to do mass capture operation for example using boar buster cages. In some states wild pig was declared vermin and hunted but limited success temporarily was achieved. The captured pigs either used for augmentation in protected areas with low ungulate densities or utilised for consumption depending on cultural and logistics constraints. Population where operation is carried out need to be monitored using camera trap-based distance sampling for demographic response to interventions used.

The nilgai population under natural conditions where 90% of females breed (adjusting for twining rate) the growth rate was 12.1%. In case when 18% of females are breeding calculated as, r = -0.179, with probability of extinction of one and $t_{1/2}$ = ~ 4 years. Under 9% of females breeding scenario in the population the intrinsic growth rate was calculated as, r = -0.287. with probability of extinction one and $t_{1/2}$ = ~ 3 years. Nilgai population control is challenging as majority of conflict areas are in a landscape with mosaic of scrub patches and agriculture with limited presence of Forest Department. The possibility of immuno-contraceptive use at large scale is not cost effective and logistically feasible in the field for nilgai. It is suggested to do mass capture operation using boma technique. In some states it was earlier declared vermin and hunted for example Bihar, but operations were not succesfull at large scale. The captured nilgai should be used for augmentation in protected areas with low ungulate densities or utilized in areas if there is cultural acceptance. It is crucial to monitor population where operation is carried out using camera trap or drone based distance sampling for demographic response to interventions.

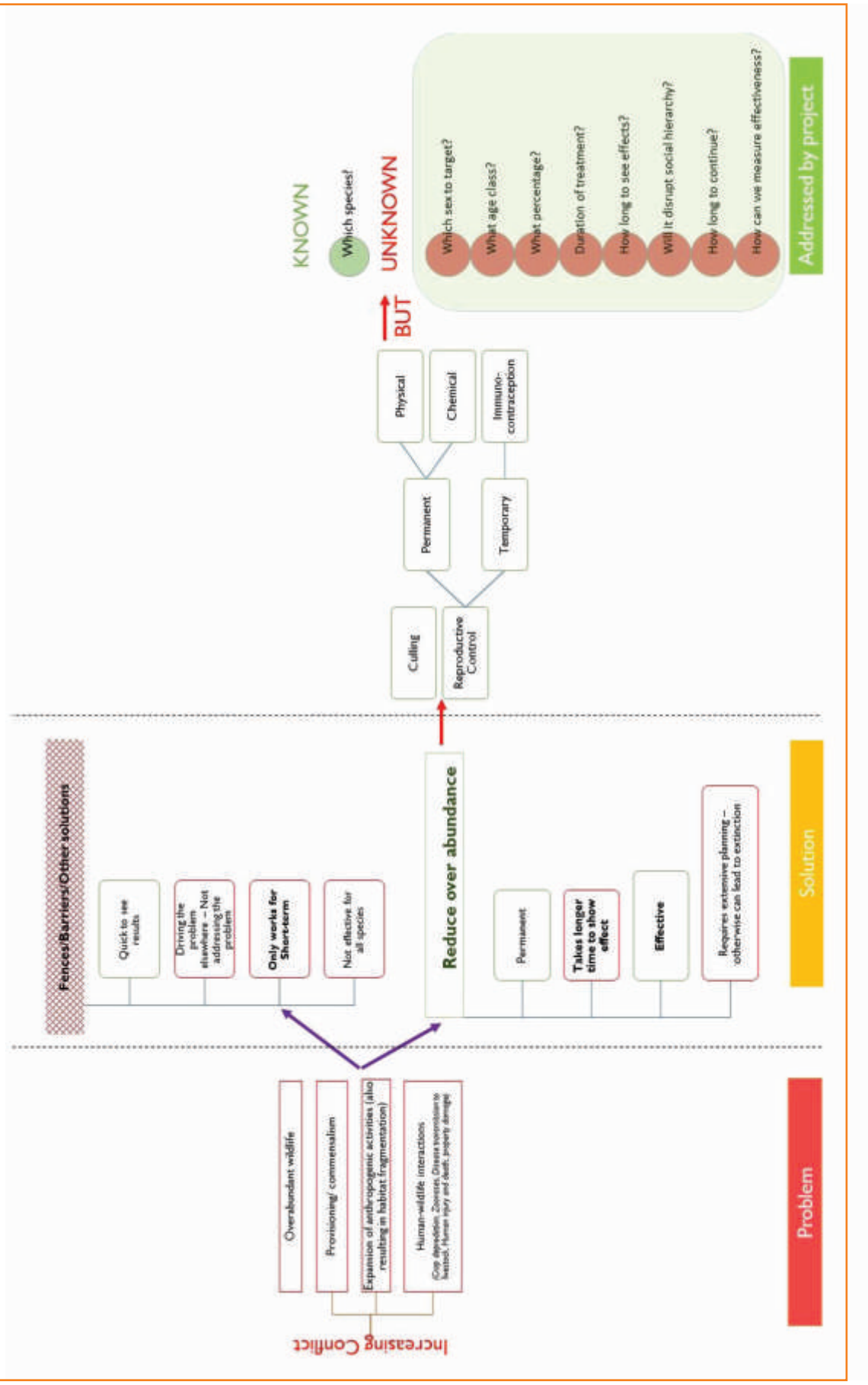
Reducing human-wildlife conflict involves adopting strategies and approaches that aim to minimize negative interactions between humans and wild animals. There is a need to protect both human livelihoods and wildlife populations by finding ways to coexist sustainably. The philosophy behind reducing human-wildlife conflict is rooted in the principles of conservation, ethics, and the recognition of the intrinsic value of both human and animal life.

The mechanisms for reducing human-wildlife conflict encompass a variety of strategies, which

can be broadly categorized into two approaches, locally mitigating conflict by using barriers to protect property, this approach focuses on proactive measures to prevent conflicts from occurring and minimizing their impact when they do happen. It includes methods like fencing, deterrents, and early warning systems to protect crops, livestock, and property from wildlife damage. Additionally, educating communities about wildlife behaviour, implementing land-use planning, and promoting sustainable farming practices can help reduce conflict. The better approach will be to do population control measures with barriers to reduce population as well as locally reduce the impact.

Overall, the philosophy of reducing human-wildlife conflict emphasizes the need for a balanced and holistic approach that considers the well-being of both humans and wildlife. By implementing effective mechanisms such as population control, conflict prevention and mitigation, and stakeholder engagement, it is possible to foster harmonious coexistence between people and animals, ensuring the long-term conservation of biodiversity while safeguarding human interests.

Pictorial Summary of Human-Wildlife conflict problems and Mitigation approaches



Chapter 1

INTRODUCTION

Qamar Qureshi, Sanath Krishna Muliya, Lallianpuii Kawlni, Vishnupriya Kolipakam, Priya Gusain, Kafil Hussain, Y V Jhala

1.1 Background

Human-wildlife conflict (HWC) currently ranks among the biggest threats to conservation initiatives across the globe and in certain cases a significant threat to health and lives of local people, the security and sustainability of their livelihoods, and to the state and national economies (Elliot et al., 2008; Benjamin-Fink, 2019). Though it is now accepted that some extent of human wildlife conflict is inevitable in human dominated landscapes, the requirement of the day is to manage the conflict in such a way that it remains within tolerable limits. Currently, a range of short-term strategies are used to mitigate HWC in India, essentially as an immediate tactical strategy to deter conflict animals. These measures are however ineffective over long period due to various reasons, including animals getting habituated to the barriers and financial burden due to ever increasing claims under compensation schemes. Thus, to minimize HWC, it is now imperative to develop new strategies, including active/invasive wildlife population management techniques.

Reproductive control methods using contraceptives have often been grouped together and described using terms such as “humane” or “benign” (Kirkpatrick and Turner, 1985, Kirkpatrick and Rutberg, 2001; Cowan and Massei, 2008; Tribe 2015) methods. The technology has been successfully used in various countries and on multiple wild animal species for active population control. For example, a review of 22 years of research on immuno-contraceptives in African elephants has successfully demonstrated the efficacy, safety, field applicability and moreover reversibility of these vaccine in small and large elephant populations in South Africa. The methodology is now being implemented in approximately 1200 females on 26 reserves across South Arica (Bertschinger et al., 2018).

There are however a number of complex technical, biological, economic and even legal issues that need to be addressed before a population control technique can be used widely in field situations. For example, the effectiveness and duration of immunocontraceptive vaccines can vary with species, age, individual differences in immunocompetence, as well as the active component of the vaccine, its formulation, dose used, delivery system and the type of adjuvant used. Thus, to investigate the feasibility and field applicability of using contraceptives as a management tool, a project titled "Population Management of Species involved in Human Wildlife Conflicts" was awarded to the Wildlife Institute of India (WII) by The Ministry of Environment, Forest and Climate Change (MoEF&CC), New Delhi, vide its order no. 8 – 98/2016 – WL dated 30th January, 2018. The project aims to develop and implement a range of HWC conflict mitigation strategies including immuno-contraception for managing certain wild animal populations in severe conflict. The project was formally initiated with hiring the services of a Project Scientists, Project fellows and a Project Coordinator in July, 2018. Further to this, various activities towards achieving project objectives were carried out in the first year of the project and a detailed report on the same was submitted to the Ministry of Environment, Forest and Climate Change, Government of India vide letter no. WII/FIN/KS/2016-2017/02, dated 14th March, 2019.

1.2 The Approach

A standard approach during any population management programme typically involves the following steps in sequential manner:

Identification of the target conflict species: Wild animals are both valuable natural resources and vital indicators of a healthy ecosystem (Fagerstone, 2002). Though abundance of wildlife is desirable in general, some species may have reached undesirably high levels in human dominated landscapes or particular populations of a species may have become locally overabundant thereby causing severe human-wildlife conflicts (Fagerstone, 2002; Mathur et al., 2015). Before initiating a population control programme against a specific species, it is thus very important to come to an agreement that, the target species/ population is undeniably harmful to human wildlife co-existence and in-turn causing negative impact on the larger conservation goals for the landscape. For the current study, four focal species as identified by the MoEF&CC, Govt of India namely Rhesus macaque (*Macaca mulatta*), Nilgai (*Boselaphus tragocamelus*), Wild pig (*Sus scrofa*) and Elephant (*Elephas maximus*) have been selected for the trials at specific sites experiencing severe conflict from above species.

Assemblage of information on species biology and ecology: The feasibility of using a particular population control method in target species/population depends on a myriad of parameters including movement pattern, population numbers, sex ratios, age structure, and estimated rate of increase and mortality of the target species in study areas (Nielsen et al. 1997; Fagerstone, 2002; Bertschinger et al., 2018). Thus, development of a population control programme as a successful wildlife management tool necessitates in-depth understanding of the demography, movement ecology, conflict and reproductive ethology, as well as physiology and biology of the

species in question, and subsequent use of said knowledge to select the most suitable population control technique. For the said purpose, focal species-specific sites viz., Dehradun and Haridwar districts in Uttarakhand (rhesus macaque and wild pigs), Kodagu and Hassan districts in Karnataka (elephant and wild pigs), Chattarpur district, areas surrounding Pench and Panna tiger reserves in Madhya Pradesh (Nilgai and wild pig) have been selected so far to carry out the intensive ecological studies. Similarly, Sir Chamarajendra Zoological Gardens Mysore (nilgai), Mattigodu and Dubare camps of Karnataka (elephant), Chidiyapur rescue and transit center Haridwar (rhesus macaques) have been identified to study reproductive biology and carryout immunology studies in captivity.

Monitor damage caused by the species & determine the damage threshold in target areas: Both species specific and target site specific socio-economic factors, including the frequency and severity of conflict and ensuing economic losses, human health (physical and mental) problems and environmental effects should be appropriately assessed before using a particular population control method in target species/population. While such assessments not only help in environmentally substantiating the population control efforts, it would also help in determining the cost benefits of proposed population control method; the capacity of existing infrastructures within institutions and the administration to undertake such programmes; and the political will or constraints urging such an action (ISSG 2018). Further, the damage information caused by a species in pre and post population control measure application periods is extremely important in evaluating the effectiveness of the applied population control method/methods vs. other possible mitigation strategies.

Selection and implementation of appropriate population control method/ methods: Globally, numerous population management techniques such as trapping, harvesting or hunting have been carried out with varied success to manage overabundant wildlife (Fagerstone, 2002). Unlike these countries, such techniques have been seldomly carried out on large scale in India, both due to legal restrictions and absence of broad consensus from the community regarding usage of such techniques (Mathur et al., 2015). Other conventional management measures, such as capture and translocation, or habitat modification has not been effective to address the issue thus far. Ensuing this, nonlethal methods for population control of the conflict species is being increasingly considered. Although there are numerous non-lethal methods available now, such as contraception (permanent, reversible, short and long acting, etc), the fundamental to the success is the selection and subsequent implementation of the method in tandem to the species and site-specific need (based on observations from steps 1- 3). Based on the extensive review of literature, techniques such as Porcine zona pellucida vaccines (rhesus macaques, nilgai and elephant), surgical sterilization (rhesus macaques), chemical sterilization (wild pig) and GnRH (hormone) based vaccines (wild pigs) will be tested for their efficacy under current study.

Monitoring post application of selected population control method: Though the initial effects such as reduced rate of birthing will be immediately visible, population control method in most of the moderate to long living species would require to run for more than 10 years in order to

show the desirable decline at population level. This necessitates an intensive and long-term monitoring of both the treated target species, as well as the study sites, so as to assess the efficacy of selected population control method/methods in resolving the conflict. While the process will not only help to apprise an ecologically sound decision on whether to continue the programme, it will also help in making an economically viable decision, supported by rigorous cost benefit and risk analyses carried out over the years of post-treatment monitoring.

Evaluation of the applied population control method/methods – both qualitatively and quantitatively: Observations made during the earlier steps will help in demonstrating both the potential benefits and the negative impacts from each selected population management technique, in terms of efficacy, field applicability, cost effectiveness, possible health hazards to the study species and even environmental impact at the study sites. A well-structured evaluation process of above facts, carried out by involving all the major stakeholders and the implementing agencies will not only provide a sound and broadly accepted consensus on investment in such programmes but also contribute to the credibility and success of this approach if found practicable.

The activities undertaken using the above approach during the II reporting period (March, 2019 to June, 2021), towards fulfilling the mandates of the project are as follows:

1.3 Activities Undertaken

1.3.1 Engagement of additional project personnel and field orientation

In order to initiate the extensive demographic and behavioural studies on target species in specific landscapes, and extend the existing laboratory capabilities, additional researchers were hired from the month of September, 2019 following all the requisite formalities.

1.3.2 Administrative Procedures

Permission from State Forest Departments for field activities: Letters seeking permissions to carryout capture, handling, health assessment, collaring and PZP field trials were communicated to concerned forest departments and the project investigators and scientists visited relevant state Forest Department to consult and expedite the process. Subsequently, permissions for the same have been received from Environment and Forests Department, Government of Uttarakhand (Rhesus macaques), Karnataka Forest Department, Government of Karnataka (Elephants and wild pigs), and Forest Department Madhya Pradesh, Government of Madhya Pradesh (Nilgai and Wild pig). Additionally, permissions to initiate the captive trails on nilgai has also been secured from Zoo Authority of Karnataka and Central Zoo authority. Further to this, the macaque studies in the state of Uttarakhand have been continued and field activities in Karnataka and Madhya Pradesh have also been initiated, enabling commencement of demographic and behavioural studies to rest of the focal species viz., nilgai, wild pig and elephants.

1.3.3 Permission for vaccine procurement and field trials

Further to the procurement of import license from the Directorate General of Health Services, New Delhi, Two Hundred doses (200) of PZP contraceptive vaccine (100 µg each) were imported from the Science and Conservation Centre, Zoo Montana, USA. However, since the vaccine is of animal origin and novel to India, several other permissions were necessary before the field trials could be initiated. Subsequently, all necessary permits for field trials, including No objection certificate (NOC) from Animal Husbandry Ministry, New Delhi; NOC from Directorate General of Health Services, New Delhi; Vaccine safety certificate from National Institute of High Security Animal Diseases, Bhopal were obtained for Macaques, whereas permits for rest of the species are still being processed by above institutions.

1.3.4 Memorandum of Understanding with Humane Society International (India)

Since the PZP vaccine, as well as raw materials to produce them are unavailable in India, MoU was signed with the Humane Society International (HSI, India), so as to procure actual vaccine for initial trials, as well as obtain the technical expertise for future local production. The HSI and their sister concerns in South Africa and USA have been extensively involved in the research which demonstrated the PZP vaccine to humanely and effectively control reproduction in African elephants and have technical knowhow on vaccine production, as well as its field implementation through their collaboration with Dr Henk Bertschinger's laboratory at the University of Pretoria, South Africa.

1.3.5 Establishment of Laboratory and Animal Housing Facilities at WII

Apart from field work, implementation of immunocontraception also involves extensive laboratory analysis. In order to assess efficacy and safety of immunocontraceptives, pre and post immunization monitoring of female animals will be performed. This experimental plan will include detection of reproductive cyclicity in females via estimation of reproductive hormones in collected faecal and blood (serum) samples by using commercially available enzyme immunoassay kits, collection of samples performed frequently at fixed intervals. Also, anti-PZP antibody titres will be estimated by ELISA to investigate the titres corresponding to induction of contraception.

In order to develop an advanced 'Wildlife Physiology & Disease Ecology Laboratory', the project team visited various animal housing and laboratory facilities including National Institute of Immunology, National Institute of Brain Research, Indian Veterinary Research Institute and Tamil Nadu Veterinary and Animal Sciences University. Based on the visits and expert inputs, a comprehensive building plan was developed. The Laboratory & Clinic building process has been completed and currently undergoing the process of furnishing with appropriate laboratory equipment, the construction work of animal enclosures is complete as well (Figure: 1.1-1.4).

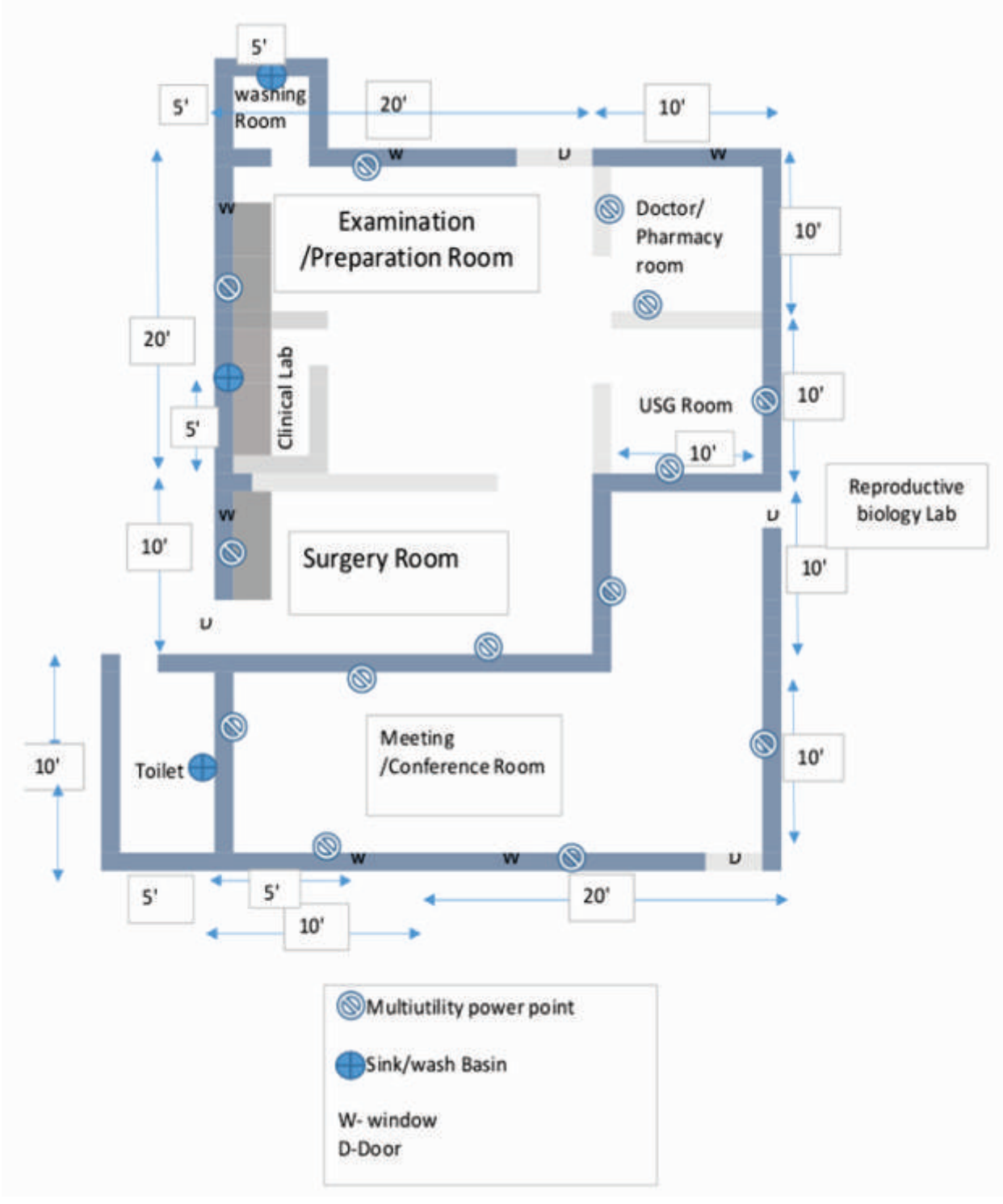


Figure 1.1 : *Layout of Veterinary Clinic Adjacent to Wildlife Physiology & Disease Ecology Laboratory*

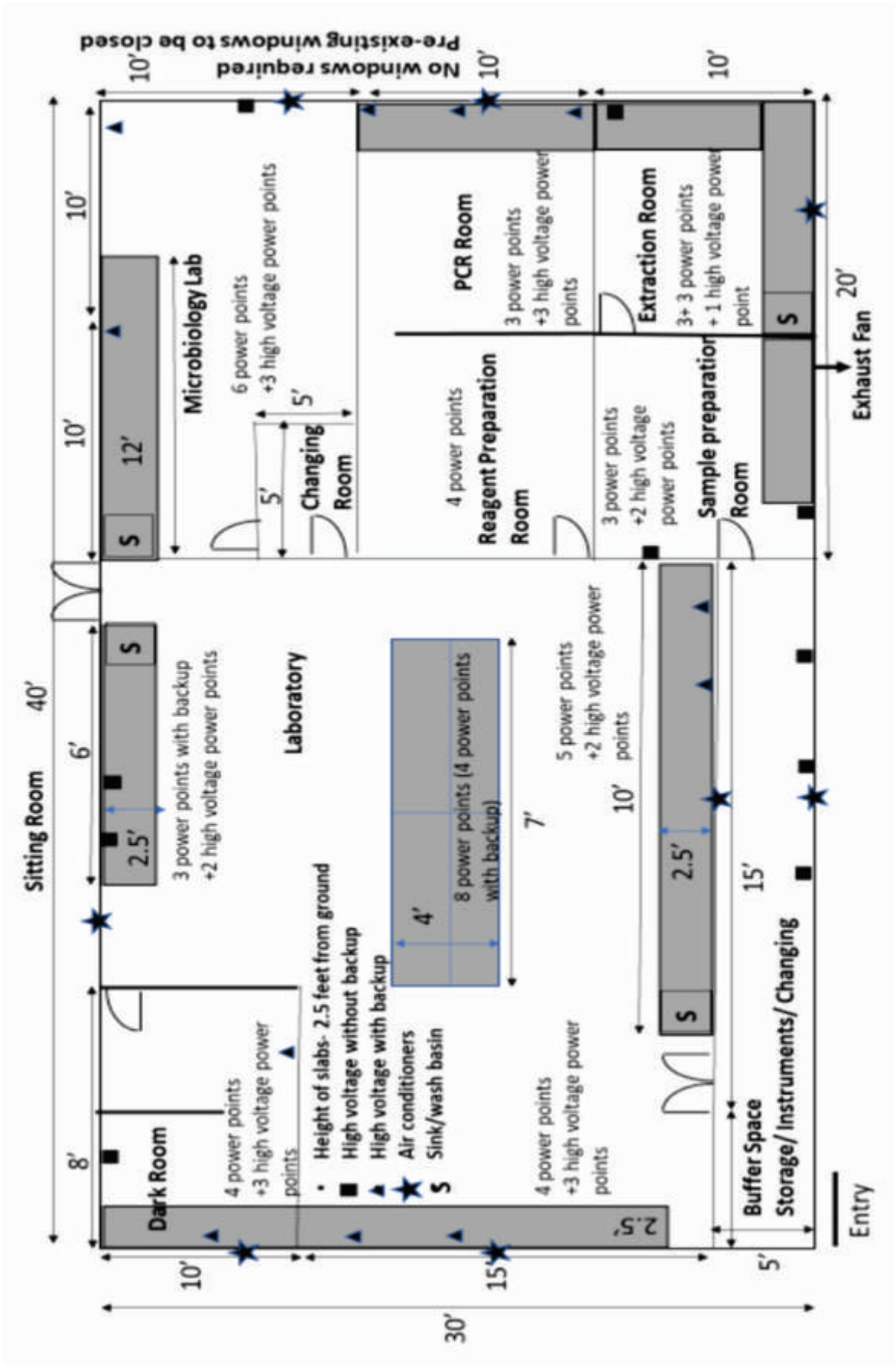


Figure 1.2 : *Layout of Wildlife Physiology & Disease Ecology Laboratory.*

Figure 1.3 : *Wildlife Physiology and Disease Ecology laboratory, Wildlife Institute of India, Dehradun.*

Figure 1.4 : *Animal Enclosure Facility associated with Wildlife Physiology and Disease Ecology laboratory at wildlife Institute of India, Dehradun.*

Inauguration of Wildlife Physiology & Disease Ecology Laboratory and Animal Housing Facilities at WII by Shri. Bhupender Yadav, Hon'ble Minister for Environment, Forest & Climate change, Govt. of India on 28th April 2022.

Glimpses of Wildlife Physiology & Disease Ecology Laboratory inauguration by Hon'ble Minister MoEFCC

SECTION I

Rhesus macaque

(Macaca mulatta)

Chapter 2

UNDERSTANDING HUMAN-MACAQUE CONFLICT (POPULATION DENSITIES AND RANGING PATTERNS IN AN ANTHROPOGENIC LANDSCAPE)

Uddalak Tathagato Bindhani, Deepika Boora, Sanath Krishna Muliya, Sarvesh Kumar, Sayli Suresh Sawant, Supravat Mahata, Divya Dwivedi, Chandrapratap Singh Chandel, Yati Gairola, Divya Ramesh, Vishnupriya Kolipakam, Lallianpuii Kawlni, Kafil Hussain, YV Jhala, Qamar Qureshi

2.1 Human-Macaque Conflict

To primate researchers across the world, the Rhesus macaque (*Macaca mulatta*) population of India is an archetypal non – human primate population, as an old-world monkey species with close evolutionary relatedness to humans among the approachable animal models for both medical, ecological as well as cognitive studies (Southwick et al. 1965; Bennett et al. 1995; Tomasello and Call 1997); usually with multimale-multifemale troop organization (Fooden 2000) primarily because of two main reasons. Firstly, the inherent ability of Rhesus macaques to successfully adapt to a varied type of environment, both natural and anthropogenic; and secondly, the changing dynamics of the traditionally recognised Rhesus – human relationship under the aegis of the constantly shifting ecological, demographic, economic and cultural forces. The ever-increasing trends in human population and developmental activities have pitted man and animals, especially non – human primates like the Rhesus macaque, to survive in overlapping habitats where humans are part competitor; part predator and part host (Richard et al. 1989). Across landscapes and habitat, the Rhesus macaque stands out as a sturdy and resilient species successfully inhabiting a wide range of forest types, agricultural areas, villages, roadsides, parks, temples and limited areas of the urban landscape (Fooden 2000; Mitra 2011; Southwick and Siddiqi 2011). The species is also intricately intertwined with the

cultural and religious beliefs of the majority of the people in India, with Hanuman being a very popular deity in Hinduism and thus, these monkeys have customarily come to be revered and worshipped across the region (Lutgendorf 2007).

Widespread deforestation is dramatically changing habitat and food resource composition through converting primary forest into mosaic landscapes of mixed agriculture and human habitation interspersed with patches of remnant forests (Achard et al. 2002). Cultivation and pastoralism open up a spectrum of new habitats. The appearance of these new ecological zones would bring about a replacement of climax forest dwellers by species that not only prefer early successional vegetation but also manage to live in close proximity to people (Richard, 1985). Some primate species are naturally more adaptable to anthropogenic disturbance. Richard and co-workers (1989) thus define the Rhesus macaque (*Macaca mulatta*), the bonnet macaque (Macaca radiata), the toque macaque (Macaca sinica) and the long – tailed macaque (Macaca fascicularis) as weed species, a separate ecological subdivision, construed as human camp followers that may even occupy some habitats only because human disturbance is present. The process of 'habitat preparation' by people may even have opened up areas previously unoccupied by Rhesus macaques. The ecological subdivision of Richard et al. (1989) predisposes Rhesus macaques to expand their ranges to include anthropogenically disturbed or modified landscapes, so as to exploit early successional plants. The similarities of human food resources (crops, garbage dumps etc.) and early successional patches thus draws our attention towards certain traits and pre-adaptations that these species exhibit. As edge specialists of interfaces between natural forests and human – modified landscapes, they have traits that preadapt them to exploit human resources (Fooden 2000; McLennan et al. 2017).

Increased human induced pressure mostly in the forms of intensified agriculture in rural areas and the increasing intolerance towards the species in urban localities (Siddiqi et al. 1988; Beisner et al. 2015); with the combined effect of rapid human population growth, forest destruction, fragmentation and conversion has led to an increase in interactions between humans and Rhesus macaques (Southwick and Siddiqi 2011), the long term effects of which on the society and behaviour of Rhesus macaques still remain unexplored. Humans and other nonhuman primate species have shared ecosystems for millennia, especially in many areas of Asia, South America and Africa (Harrison 1996) with researchers having observed certain primate species thriving under the scenarios of anthropogenic habitat modification (Asquith 1989; Richard et al. 1989; Strum 1994). The commensal tendency of Rhesus macaques to adapt to human villages, towns and cities and other landscapes, subject to anthropogenic modification (Seth and Seth 1983; Richard et al. 1989), have aggravated agonistic interactions between man and Rhesus ensuing in human – wildlife conflicts via raiding of agricultural fields and homes. The other result of such frequent interactions is the purposeful feeding of Rhesus by humans or the chance availability of anthropogenic food sources in garbage dumps.

Human disturbance has important consequences for the biology of primates. The frequency of contact between humans and monkeys, the degree of overlap in space, the degree of commensality, and the amount of time primates spend in areas containing human-generated food are all of biological significance (Strum 1994). This is likely to give rise to shifts from existing

behavioural patterns of ranging, foraging pattern, inter and intra specific interactions, migration and reproduction, in the short term with adaptations and variations to evolve in the long term for Rhesus troops (Bishop 1981; Asquith 1989). Provisioning and supplemental feeding, whether in the form of crop raiding, tourist handouts, or active provisioning, has the potential to be one of the most influential forms of anthropogenic disturbance, affecting primate ecology, demographics and social behaviour (Sinha et al. 2005; Kaburu et al. 2019). The act of provisioning by humans and the primate's active supplementation of natural foods by access to anthropogenic food resources (raiding and scavenging) lead to altered quantity or composition of their diets leading to important consequences in their innate biological characteristics (Hill 1999; Strum 2010). Such variables might predispose resilient primate species like the Rhesus macaques to respond by undergoing subtle but rapid changes in their behavioural ecology.

Recent rise in human – Rhesus conflicts have been primarily attributed to a rapid population growth of the Rhesus macaques across the Indian subcontinent (Imam and Ahmed, 2013; Singh et al. 2016). Spurt in incidences of crop and home raiding coupled with occasional serious attacks on humans by Rhesus have led to the implementation of mass sterilization, translocation and culling projects in certain parts of the country (Rattan, 2011). Previous demographic and abundance studies for Rhesus though have reiterated the rise in macaque populations (Southwick et al. 1994; Southwick and Siddiqi 2011), the exact ecological factors and socio-behavioural parameters leading to this rise are still a matter of great deliberation (Southwick and Siddiqi 2011; Imam and Ahmed, 2013; Singh et al. 2016).

The long-term sympatry between man and non – human primates can create a complex web of behavioural, ecological, epidemiological and economic relationships. The effects on Rhesus macaque troops in human dominated or modified landscapes with respect to the dynamics and interplay of population growth rates owing to natality, infant mortality, and inter - birth intervals, secure important parameters in further realising primate population dynamics under anthropogenic influences. Further, the response of the species with respect to movement and ranging in such habitats needs characterizing from the perspective of foraging efficiency and troop sizes. Increased interaction with humans and fluidity of the human-macaque interface should trigger novel patterns in behavioural patterns and activity budgets. The response of anthropogenic troops to stress due to increased competition, aggression (humans and conspecifics) and agonistic interactions in a despotic contest competition for resources, also requires investigation. Amalgamation of these behavioural and demographic findings with efficient management practices shall aid in abatement of conflict and realising scientific conclusions towards the well-being of both man and animals.

2.2 Assemblage of information on Rhesus Macaque biology and ecology

2.2.1 Study Area

The study is being carried out in the 5 km radius of Wildlife Institute of India, Uttarakhand, which includes a mixed matrix of human habitation, farm lands, urban forest patch and a national park. The area of 16sq.km. around Wildlife Institute of India is also an area of high intensity of human use, having open sewage and ample garbage dumps, all of which have acted as attractants for macaques to colonise the area. Macaques here are known to feed on subsidies provided by humans, interact with livestock, dogs, and humans, thereby creating a potential for conflict in the interface.

2.2.2 Population status and demography of Rhesus Macaques

Distance sampling techniques of trail or line transect surveys are widely employed across various habitat types to obtain population density estimates of primates. Distance predicts population density by determining a detection probability function of observing an animal at increasing perpendicular distances from the transect line, and correcting for observations missed by testing data against several models to establish effective strip width (Buckland et al. 2001; Buckland et al. 2010).

The population density of *Macaca mulatta* was investigated by conducting trail-transect surveys across the 16 sq. km study area, which comprises of a mosaic of forested (sal plantations; mixed forests) and anthropogenic (human habitation, agricultural fields, roads) habitats. The study area was divided into sixteen square grids of 1 sq. km each. Existing trails in the forested patches and roads/lanes through the human-modified landscape were used to lay 8 transects (7 transects of 2 km each; 1 transect of 1.5 km; total transect length of 15.5 km) throughout the study area, representing every 1 sq. km grid, encompassing the study site.

The transect walks were conducted across seasons, taking into consideration the ecology of and behaviour (breeding season, birth of infants, inter-troop migration of individuals etc.) of

the Rhesus macaques. Each transect was sampled thrice within a day, by a team of two trained observers, via temporal replicates (06:00 AM-12:00PM-04:00PM). A total survey effort equalling 46.5 km was made within a season during each transect sampling exercise. Rhesus macaques are social animals with socially distinct troops and are thus encountered in clusters. A datasheet was maintained for each transect replicate to record the following parameters: date, time, replicate, transect name, GPS coordinates of the start and end point and region/landmark of the transect, time of each sighting, number of animals encountered, GPS location of sighting, radial distance of the encountered individual/cluster, angle of sighting, major habitat/substrate where animals were encountered. A laser equipped range finder, magnetic compass and electronic GPS were utilized to record distance of sighting (in metres), angle of sighting (in degrees) and geographical coordinates (in degree-decimals).

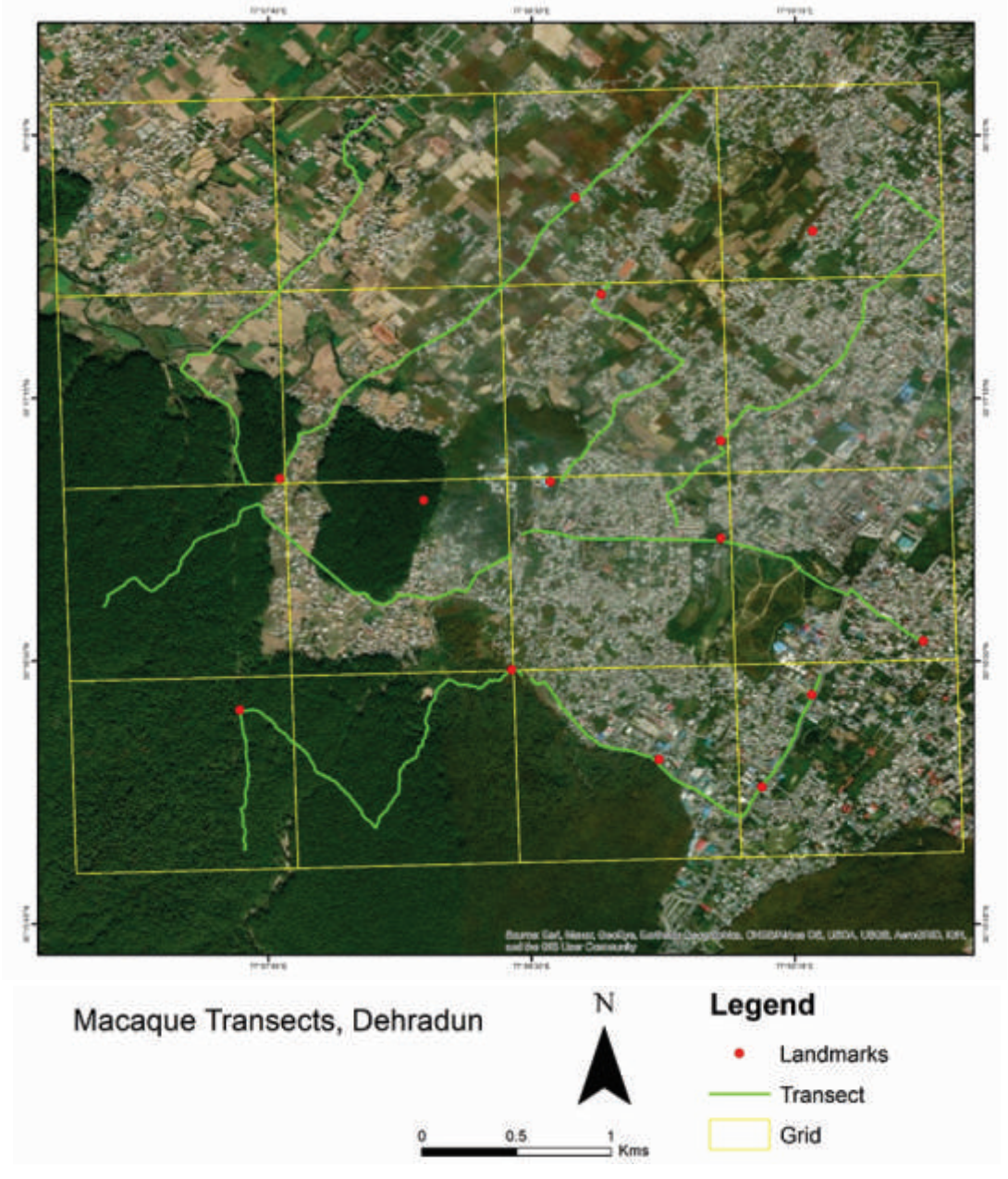


Figure 2.1 : *Trail transects in the study area (Chandrabani, Dehradun).*

The obtained distance sampling data were subjected to analysis with the R Distance package on statistical software R and DISTANCE 7.0 using post-stratification by season. Outliers were removed via preliminary data visualization. All observations beyond 70m (radial distance) were truncated. The corrected Akaike Information criterion (AICc) was used to select the best-fit model. The half-normal model with cosine adjustments was selected with the lowest AICc value of 1278.58. The detection probability was 0.26 while the effective strip width (ESW) was 17.86±0.94m. The overall densities calculated during the study period predicted the presence of ~143 macaques per square kilometre.

A low density estimates in December 2020 and April 2021 (post-breeding; initiation of parturition) could be attributed to the low number of sightings during the season, possibly due to changes in troop foraging patterns due to the lack of provisioning during the ongoing COVID19 pandemic. Reported densities for Rhesus macaques vary from 32.11 individuals per sq. km in forested landscapes to 201.1 individuals per sq. km in non-forested habitats (Fooden 2000). The high standard errors are possibly due to the clumped distribution of the species

Table 2.1 : Density estimates obtained from transect data analysis in the study area around WII.

Month/Season	Effort (km)	Groups encountered	Mean Group Size	Density	95% Confidence Interval	Encounter Rate
August 2020 (Monsoon)	46.5	31	4.61±0.83	124.06±55.21	49.12-313.34	0.67
December 2020 (Breeding)	46.5	22	7.00±1.65	78.32±47.99	22.87-268.23	0.47
April 2021 (Parturition)	46.5	20	5.05±1.08	71.30±36.38	24.58-206.83	0.43
July-September 2021 (Monsoon)	93	67	6.31±0.91	141.86±57.61	59.37-338.98	0.35
November-February 2021-2022 (Breeding)	93	72	5.21±0.67	118.28±51.57	46.28-302.22	0.49
May-June 2022 (Parturition)	93	42	8.36±1.18	133.63±53.71	58.69-304.27	0.45
September-October 2022 (Monsoon)	93	48	11.33±1.72	237.7±88.45	112.91-500.51	0.52
January-February 2023 (breeding)	93	73	9.01±0.96	235.51±83.53	109.6-506.06	0.78
Overall				142.59±23.67 (~143 per sq. km)	103.96-195.56	

Table 2.2 : Rhesus macaque age-sex class ratios observed in study area around WII.

ASC ratios	August 2020	December 2020	April 2021	July 2021	September 2021	November 2021	February 2022	June 2022	October 2022	February 2023
AM:AF	1:1.5	1:1.43	1:2.31	1:1.49	1:2.11	1:1.88	1:2.23	1:2.17	1:2.27	1:2.21
INF:AF	1:1.08	1:1.43	1:1.95	1:1.04	1:1.48	1:2.35	1:2.41	1:1.68	1:1.42	1:1.99
AM: JUV	1:1.62	1.09:1	1:1.63	1:1.46	1:1.97	1:1.8	1:1.85	1:2.51	1:2.56	1:3.07
AF: JUV	1:1.08	1.56:1	1.42:1	1.02:1	1.07:1	1.04:1	1.20:1	1:1.16	1:1.32	1:1.39
JUV:Adults	1:1.55	1:2.66	1:2.04	1:1.7	1:1.57	1:1.6	1:1.74	1:1.26	1:1.27	1:1.04

while anthropogenic structures in the landscape impede animal sightings. The age-sex class sex ratios were calculated as per the sightings made during the transect exercises (AM: Adult ♂; AF: ♀; JUV: Juvenile; INF: Infant). Comparatively similar numbers of males and females were encountered during the transects while the INF:AF ratios were high, across seasons. Similar trends were observed for Rhesus troops in Dehradun by Seth and Seth (1983).

2.2.3 Standardizing methods for abundance estimation of Rhesus macaque using Genetic mark recapture methods

For rhesus macaques, initial survey techniques were basic and usually involved trying to approximate a complete count of the rhesus population, and current population estimation techniques generally involve "line transect" survey methods (Southwick et al. 1961a; Southwick et al. 1961b; Seth and Seth 1983; Southwick et al. 1983; Southwick & Siddiqi 1988; Tiwari and Mukherjee 1992; Pirta et al. 1997; Plumptre and Cox 2006; Chaudhuri et al. 2007; Murmu et al. 2011; Southwick and Siddiqi 2011; Imam and Ahmad 2013; Jaman and Huffman 2013; Kumar and Anita 2015; Singh et al. 2016; Prasad et al. 2018; Anderson et al. 2019). In direct count methods, distance sampling is often employed and evaluation of detection probabilities based on proximity and visibility to the observer are needed to extrapolate observations into population abundance estimates (Plumptre et al. 2013), and that may differ from observer to observer (Mitani et al. 2000). As these estimates based on the extrapolations and assumptions are liable to suffer from low precision, monitoring and comparisons over time to assess population abundance changes are highly problematic (for example; Roy et al. 2014b).

In recent years, with easy accessibility and advancement of molecular techniques, the use of genetic tools to survey primate populations is being implemented under two general approaches: (i) the analogous of a total count survey where individuals are identified by genotyping DNA extracted from hairs (Goossens et al. 2005) or fecal samples (Guschanski et al. 2009) and (ii) genetic capture-recapture analysis (Arandjelovic et al. 2011; Hickey & Sollmann 2018). Genetic capture-recapture method is very new for primate population estimations and is still being tested. Although, it has been implemented for the past 10-15 years quite effectively for coyotes (Kohn et al. 1999), elephants (Eggert et al. 2003), bears (Coster et al. 2011), Eurasian otters (Lampa et al. 2015) and sage grouse (Shyvers et al. 2020), etc. Genetic capture-recapture methods are mainly based on traditional mark-recapture methods. In traditional mark-recapture methods, individuals from the population are captured physically, marked with a unique ID tag, and then released back into the population. Researchers re-sample the population multiple times and identify marked individuals from a previous capture. The time interval between two successive sampling sessions is such that marked individuals get re-shuffled completely into the population. A capture history is created for each captured individual showing, for each sampling session, whether the individual was captured or not captured. The capture histories of every captured individual are recorded into a capture history matrix. There are many models with varying assumptions to evaluate capture history matrices (Williams et al. 2002). With fulfilment of model assumptions, mark-recapture models can give

accurate estimates of population abundance and size. In genetic capture-recapture methods, an individual is uniquely identified by genotyping its DNA using a set of molecular markers, and its genotype considered as its DNA tag. When the same genotypes is detected in two different samples, the 2nd sample is regarded a “recapture”. Physical capture can cause injury to an animal or can be stressful, decreasing the survival ability of animal in the wild (Langer 2006), or it can cause changes in animal behaviour which in turn effect the estimates of population size (Williams et al. 2002). Employing non-invasive genetic sampling in genetic capture-recapture methods can solve these problems.

In the current study area of 16 sq. km around the Wildlife Institute of India, Dehradun, we aimed to standardize methodology for genetic based mark-recapture of macaques for abundance estimation. Abundance estimation method using genetic mark-recapture will be tested against line-transect based estimates to understand accuracy and precision of these methods. The current study area is divided into sixteen grids of dimensions 1 sqkm. For sampling, polygon search method has been used. Total 8 sessions of trail survey have been conducted from June 2021 to March 2022, to collect the rhesus faecal samples. During each sampling session, each grid was searched intensively to maximize the area coverage and sample size. Every grid is searched only once in a session, swiping from one end to another, keeping a GPS track of survey paths walked. Total 1078 faecal samples (with information of sample's GPS coordinates, date and time of collection) have been collected during these 8 sessions.

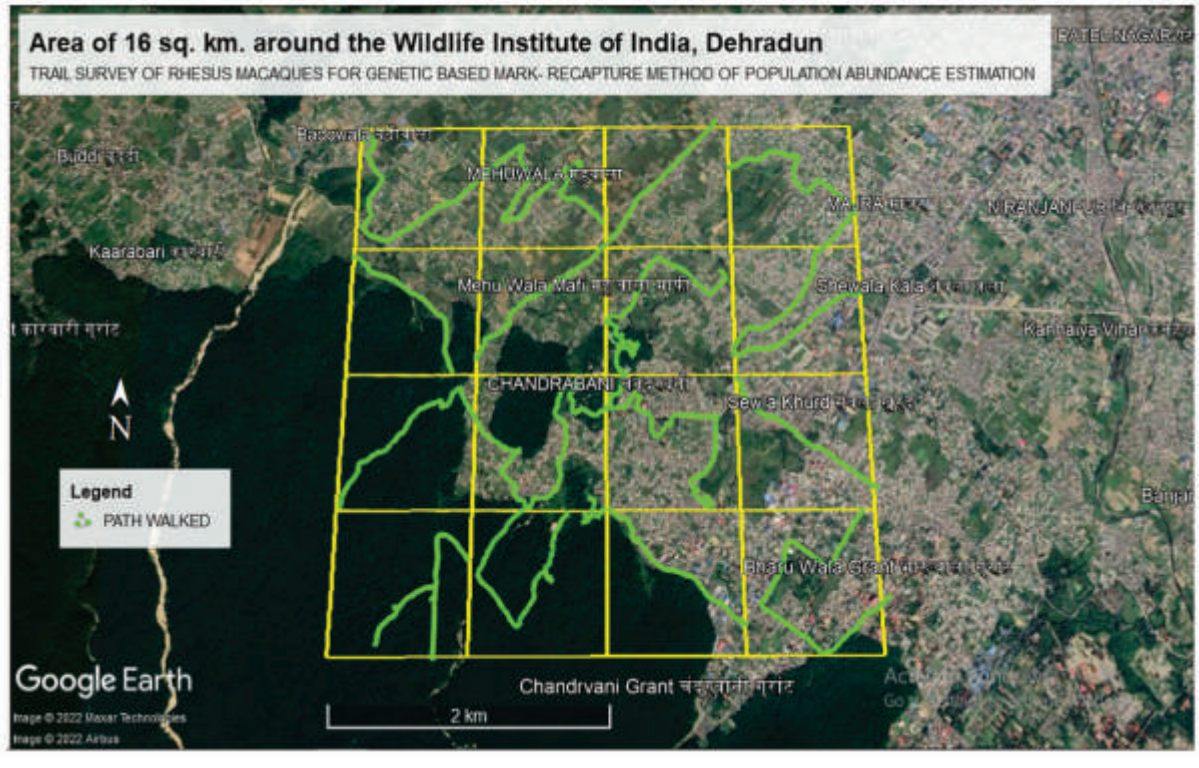


Figure 2.2 : *Trail survey of rhesus macaques for genetic based mark- recapture method of population abundance estimation*

Table 2.3 : Session-wise sample collection during trail survey of rhesus macaque

Session	No. of Faecal Samples
Jun-21	34
Aug-21	30
Sep-21	14
Oct-21	57
Nov-21	180
Dec-21	178
Feb-22	408
Mar-22	177
Total	**1078**

DNA extraction protocol has been standardized for faecal samples using GuSCN- Silica based method (Boom et. al. 1990). 493 faecal samples have been processed till date. For species identification of faecal DNA samples, Rhesus macaque species-specific primer has been designed by targeting the Mitochondrial CYTB region. For sex identification of the Rhesus faecal DNA samples, we have to select appropriate sex-linked molecular primers which target both sex-chromosome markers, such that ambiguity of false negative or false positive samples can be solved. So, we have selected X and Y-chromosome linked markers from available literature. The primer for these markers is being standardized. For individual identification, microsatellite genotyping is being used for Rhesus faecal DNA samples. We have selected 20 microsatellite

Table 2.4 : Details of microsatellite marker panel for individual identification of Rhesus macaques. This panel will be narrowed down to 10-15 markers after further testing.

S.No.	Primer Name	Repeat Motif	Reference
1	D20S171	di	Rogers et. al. 2005
2	D12S67	tetra	Nürnberg et. al. 1998
3	D19S559	tetra	Rogers et. al. 2005
4	D5S1457	tetra	Kanthaswamy et. al. 2006
5	D7S794	tetra	Kanthaswamy et. al. 2006
6	D10S179	di	Rogers et. al. 2005
7	D1S1594	tetra	Rogers et. al. 2005
8	D8S1466	tetra	Rogers et. al. 2005
9	D4S2964	di	Hadfield et. al. 2001
10	D6S474	tetra	Nürnberg et. al. 1998
11	MML14S2	di	Raveendran et. al. 2006
12	MML16S3	tetra	Raveendran et. al. 2006
13	D16S403	di	Rogers et. al. 2005
14	D9S921	tetra	Kanthaswamy et. al. 2006
15	D17S791	di	Rogers et. al. 2005
16	D18S537	tetra	Kanthaswamy et. al. 2006
17	D10S611	tetra	Kanthaswamy et. al. 2006
18	D3S3045	tetra	Rogers et. al. 2005
19	D7S513	di	Hadfield et. al. 2001
20	D13S894	tetra	Smith et. al. 2000

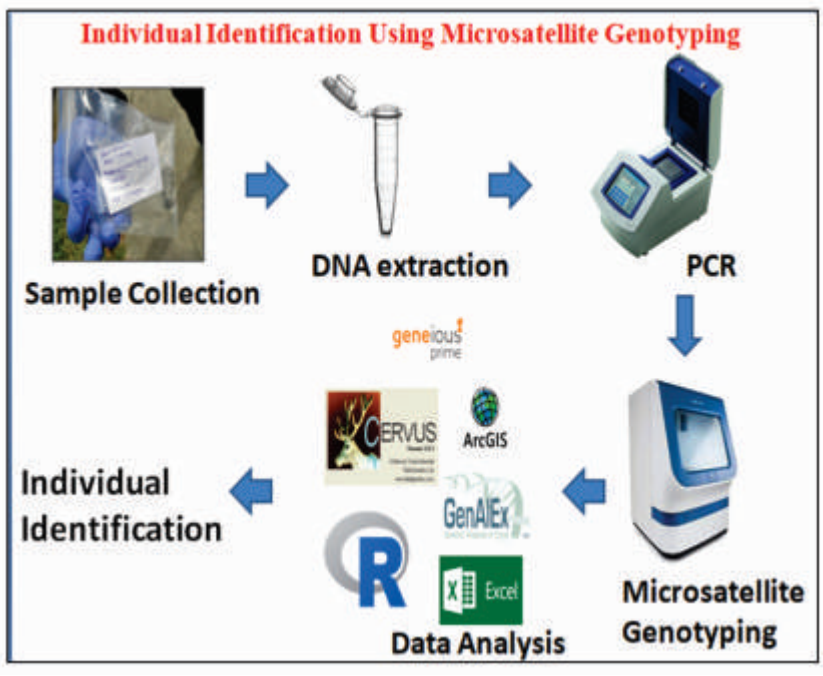


Figure 2.3 : *Individual identification using microsatellite genotyping.*

markers from available literature based on the high polymorphism, low allele size and repeat motif. Out of 20 microsatellite markers, a set of 10-15 microsatellite markers will be selected based on the PID Values and relatively easy PCR amplification procedure. Standardization of the microsatellite markers is in process.

2.3 Movement and ranging patterns of Rhesus macaque in Chandrabani, Dehradun

The Rhesus macaque (*Macaca mulatta*) is a cercopithecine Old World monkey that has emerged as an important species in the field of human-wildlife conflict in India, due to its high level of synanthropy and efficient utilization of anthropogenic landscapes. This long-term sympatry between man and Rhesus macaques has created a complex web of behavioural, ecological, epidemiological and economic relationships between the two species. A better approach and understanding of these multiple factors would lead to improved mitigation measures to deal with conflict scenarios and alleviate severity.

In view of the above and to better realise the prospects of effective immuno-contraception protocols for the species, the Wildlife Institute of India has initiated the study of Rhesus macaque troops within a 2 km radius of the campus. Four socially distinct Rhesus troops have been identified and habituated for behavioural monitoring and feeding ecology observations of the troop members. All four troops occupy and range over a mosaic of forested and anthropogenic landscapes within the designated study area of 16 sq. km. Sal plantations and mixed forests comprise the forested habitats while human habitation, agricultural fields and roads make up the anthropogenic component of their habitat. A sexually mature adult female

(associated dependant infant) from each of these four troops have been fitted with GPS enabled VHF radio collars aiding in identification, tracking, monitoring and telemetry studies.

Trap cages were setup to capture a preferred adult female from the four habituated troops within the study area. Adult females were collared to ensure long-term ranging and movement data from the collars, as they exhibit troop natality. Fruits, chickpea and groundnut were used as bait to lure the macaques into the cages. Upon the successful capture of an adult female, the animal was anesthetised and the radio collar fitted around its neck. All animal handling operations were conducted by wildlife veterinarians in concurrence with the ethical guidelines of the Wildlife Institute of India and the Dept. of Forests, Govt. of Uttarakhand.

The obtained radio collar data were subjected to analysis on R statistical packages and ArcGIS software to obtain the ranging and movement parameters for the collared individuals. The geographic coordinates obtained in the degree-decimal format were transformed to the Universal Transverse Mercator projection (WGS 1984, Zone: 44N) to facilitate calculations in the metric system.

2.3.1 Updates for the period 2018-2020

One adult female from each of the four identified and habituated socially distinct study troop was collared with Lotek GPS loggers programmed to take location fixes every hour.

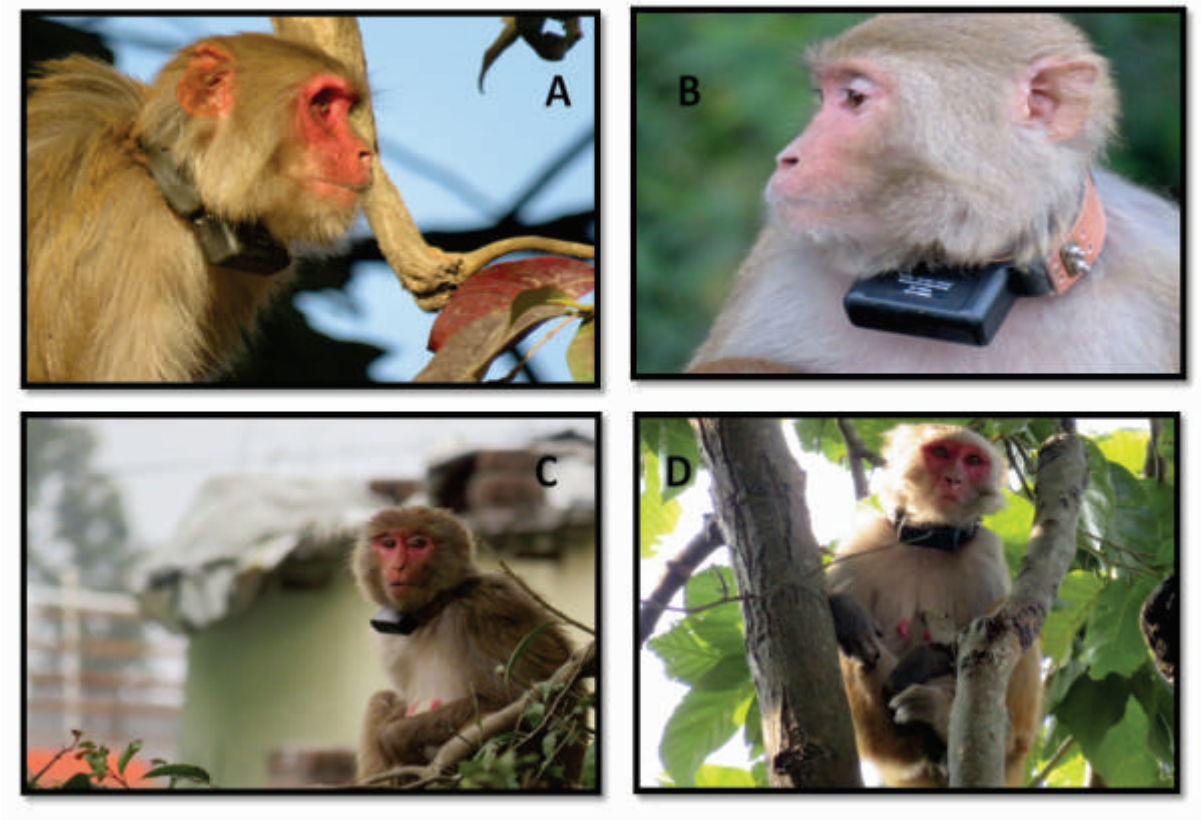


Figure 2.4 : *Photographs for all four collared adult females, depicted as per their troops.*

Table 2.5 : Summarised details of deployed collars in study area around WII.

S. No.	Troop	Age-Sex Class	Associated Infant	Collar Id	Collar Frequency (MHz)	Date of Capture and Deployment	Data Obtained (days)
1.	A	Adult Female	Yes	31041	164.040	26/05/2018	316
2.	B	Adult Female	Yes	31039	164.000	23/11/2018	220
3.	C	Adult Female	Yes	31040	164.020	24/07/2018	345
4.	D	Adult Female	Yes	31042	164.050	20/11/2018	253

Home range and Core range sizes

The Kernel Density Estimation (KDE) methods were used to determine the home ranges (95%) and core ranges (50%) of the collared females. The reference bandwidth (href) and the least-squares cross-validation bandwidth (LSCV) were utilized as smoothing parameters for the analysis (Figure 2.5-2.8). The two bandwidth parameters predicted a home range size of 19.21–70.48 hectares and a core range size of 1.99–13.67 hectares, for all four collared females. The href bandwidth predicted larger home ranges for the individuals. The core range sizes calculated via MCP were larger than that calculated by KDE, 2.21–15.42 hectares. The collared individual from troop C recorded the highest home and core ranges while troop D had the lowest values.

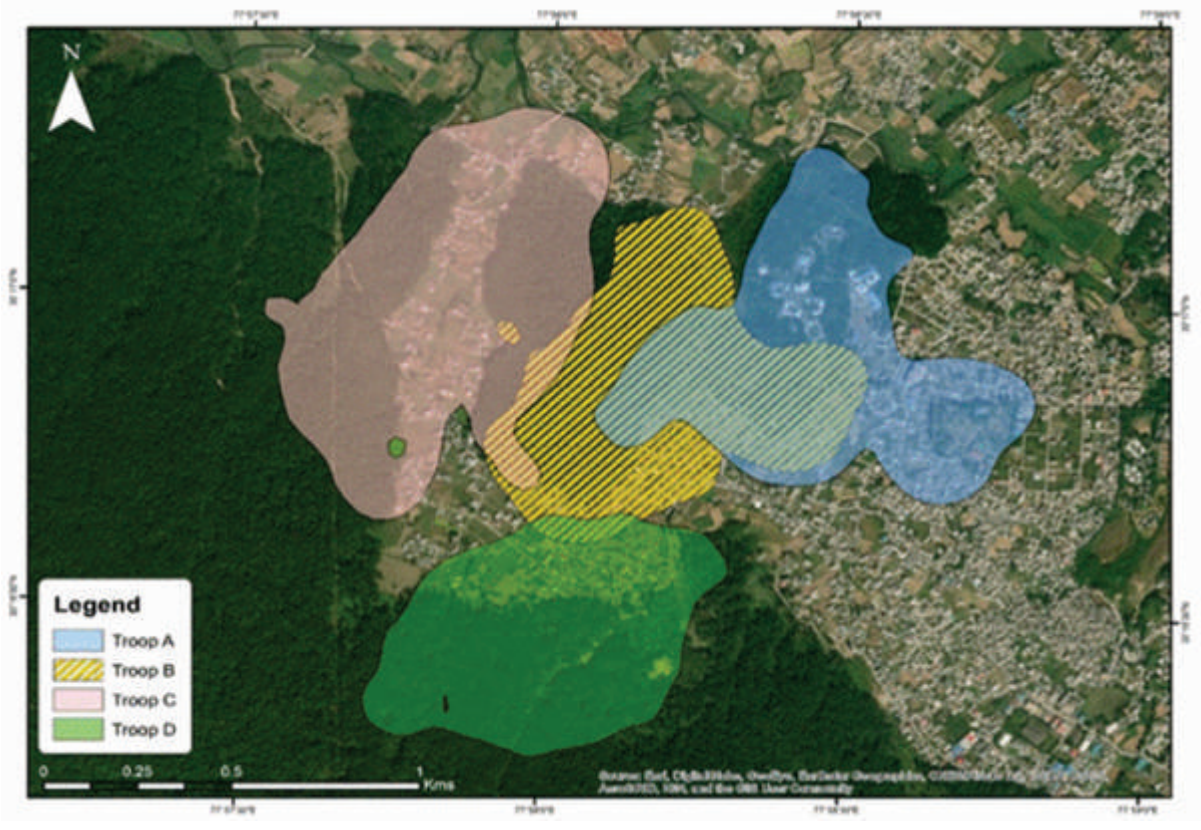


Figure 2.5 : *Home range (95% volume contours) for the four collared adult females in the study area; href bandwidth.*

Table 2.6 : Home range (95%) and Core range (50%) sizes in hectare (1 ha=0.01 sq. km=10000 m^2).

Troop (Adult Female)	KDE href 50% (ha)	KDE LSCV 50% (ha)	KDE href 95% (ha)	KDE LSCV 95% (ha)	MCP 50% (ha)	MCP 95% (ha)
A	8.18	3.58	60.57	28.55	11.18	69.43
B	8.76	4.22	57.25	33.17	10.31	59.10
C	13.67	5.68	70.48	39.64	15.42	83.85
D	3.95	1.99	49.47	19.21	2.21	41.28

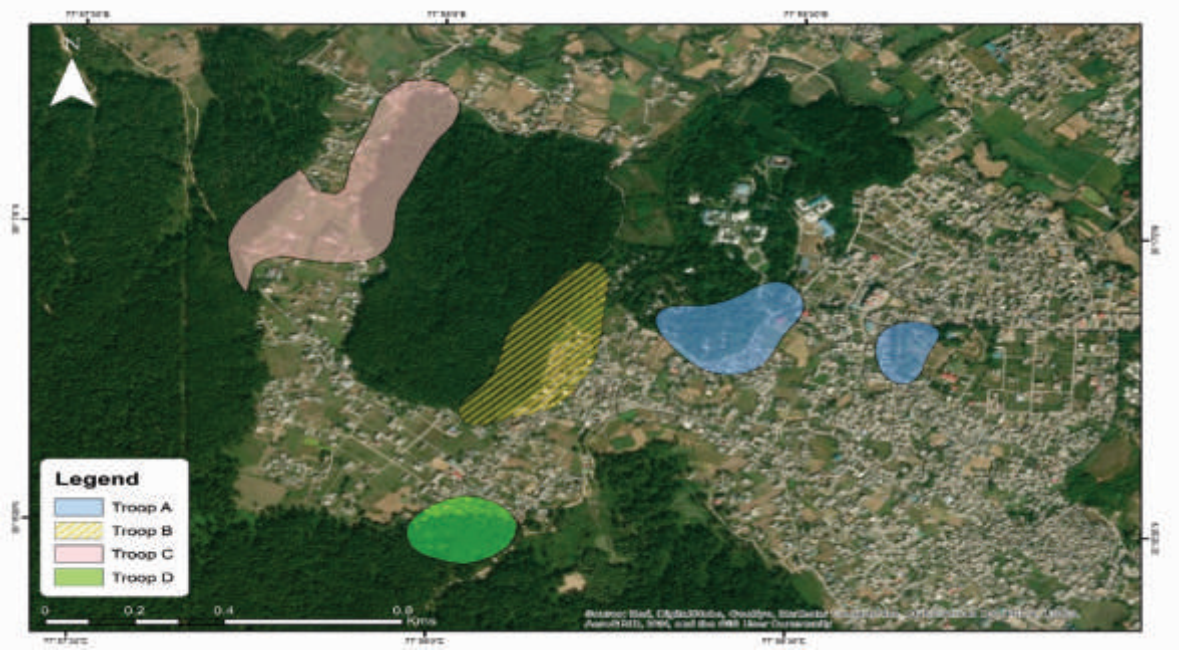


Figure 2.6 : *Core ranges (50% volume contours) for the four collared adult females in the study area, href bandwidth.*

Figure 2.7 : *Core ranges (50% volume contours) for the four collared adult females in the study area, LSCV bandwidth.*

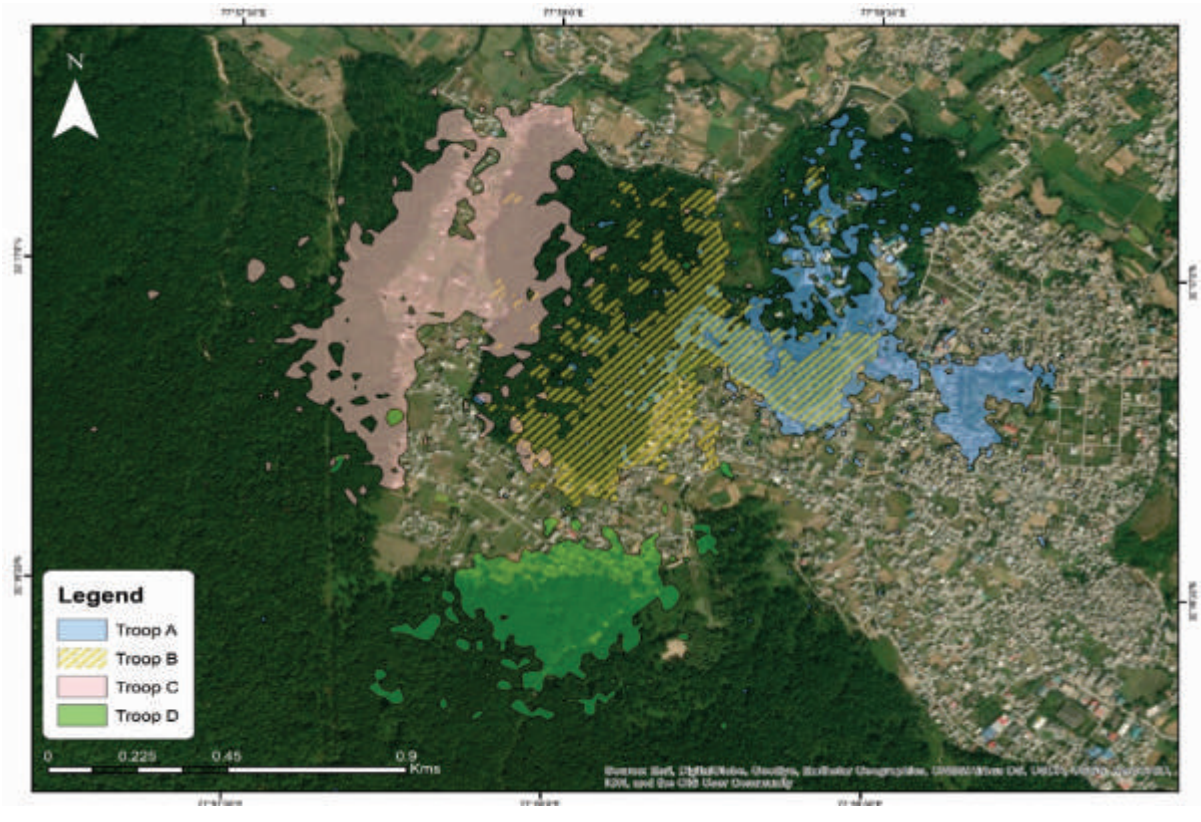


Figure 2.8 : *Home ranges (95% volume contours) for the four collared adult females in the study area, LSCV bandwidth*

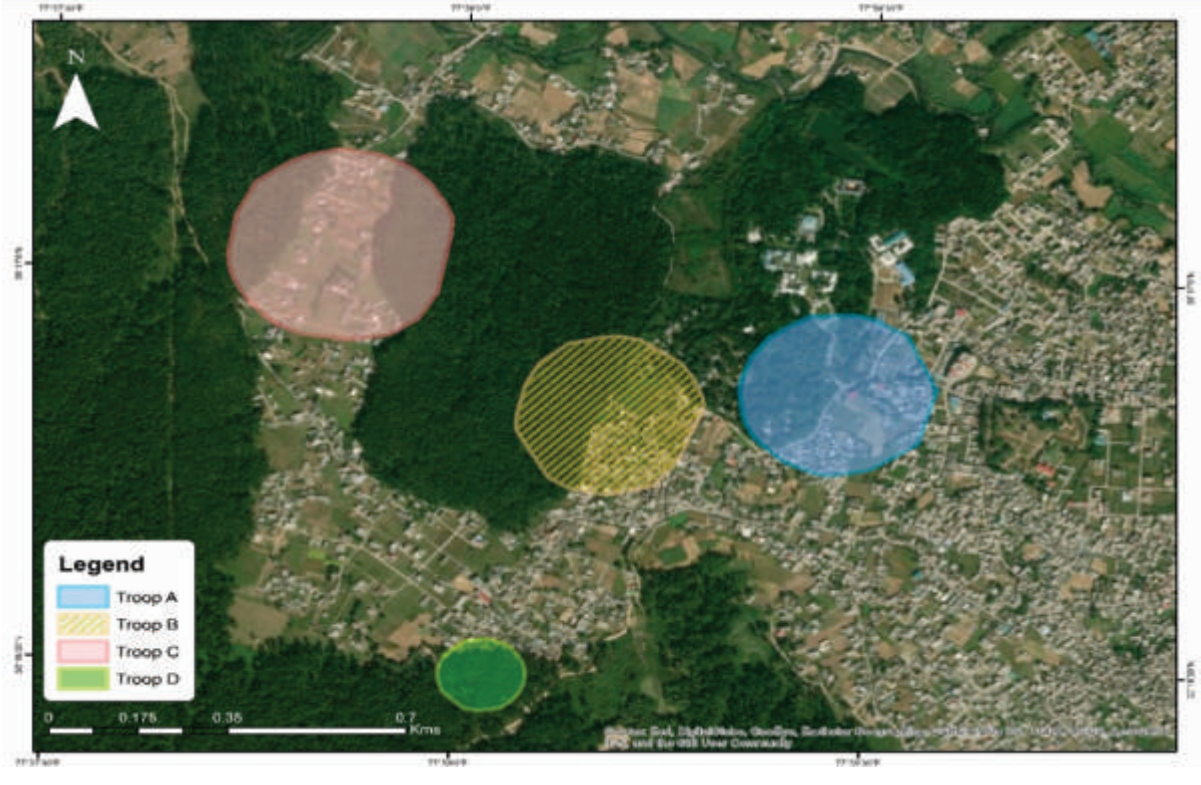


Figure 2.9 : *Core range (50% MCP) for the four adult females, Minimum Convex Polygons.*

Table 2.7 : Home range overlaps for the collared individuals.

Troops	KDE 95% href (ha)	KDE 95% LSCV (ha)
A – B	23.28	10.3
B – C	4.53	1.29
B – D	1.71	0.1
C – D	0.21	0.19

The KDE volume contours have shown varied overlap in home ranges of neighbouring troops. The 95% volume contours for both KDE bandwidths highlight a certain degree of overlap, regions being utilized by both troops. Troop B, inhabiting the sal plantation nested amidst athropogenic structures, had overlaps with all the other troops. The LSCV bandwidth also predicted a core range range overlap of 0.29 hectares between troops A and B only, for shared regions within the campus of the Wildlife Institute of India. No troops showed overlaps for core ranges predicted by the href reference bandwidth. The overlap predictions for the href reference bandwidth were higher than those obtained via the LSCV bandwidth. The core ranges calculated via MCP did not predict any overlap between the four collared adult females.

Collared animal travel and movement

- Total Daily Movement

The mean total distance covered in a day ranged from 1.54 km to 2.1 km for all four study troops.

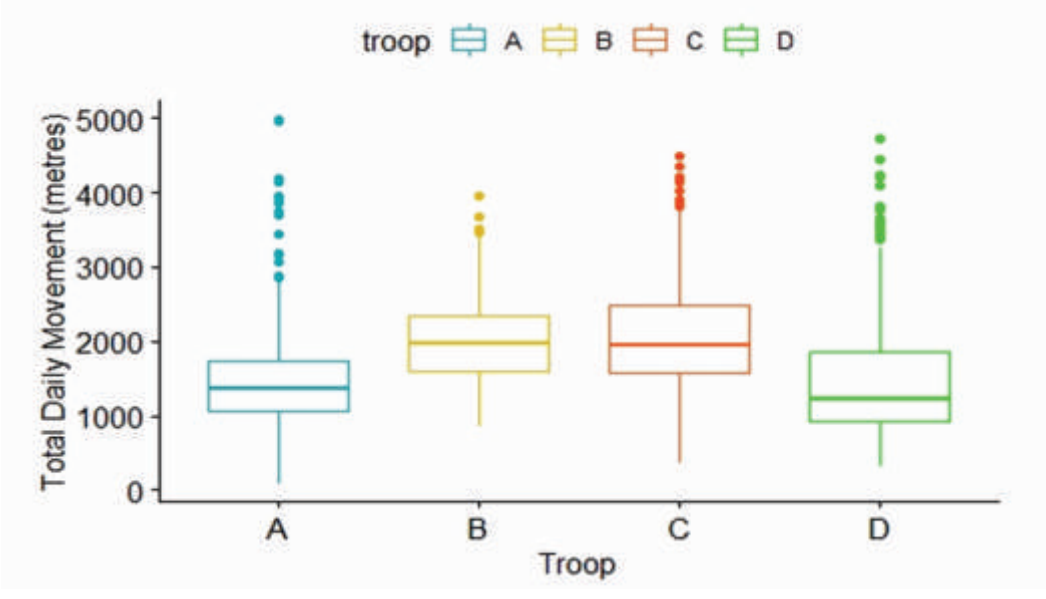


Figure 2.10 : *Troop wise data visualization for collared individual's total daily movement.*

Table 2.8 : Summarised daily movement statistics for the collared macaques.

Troop	Count (days)	Mean ± S.E. (metres)	Median (metres)
A	312	1534 ± 41.16	1362
B	220	2029 ± 40.31	1979
C	336	2092 ± 38.35	1954
D	247	1536 ± 56.95	1222

The adult female from Troop C recorded the highest mean total distance covered on a daily basis at 2.09 km (N=336) while Troop A had the lowest mean daily distance at 1.53 km (N=312). Troops B and D recorded average daily movements to be 2.03 km (N=220) and 1.54 km (N=247) respectively.

The mean ranks for the total recorded daily distance for the four collared adult females showed significant differences to exist between them (H=208.99, df=3, $p < 0.001$). A pairwise comparison amongst the four collared individuals showed no difference between troops A and D (p=0.066). Troops B and C too showed no difference between them (p=0.606). All other pairs were found to be significantly different from each other (p<0.05).

- Daily Displacement

The daily displacement for each collared rhesus macaque was obtained by calculating the straight-line distance between the first and the last geo-coordinate fixes for a day. The mean daily displacement was then computed for all four individuals.

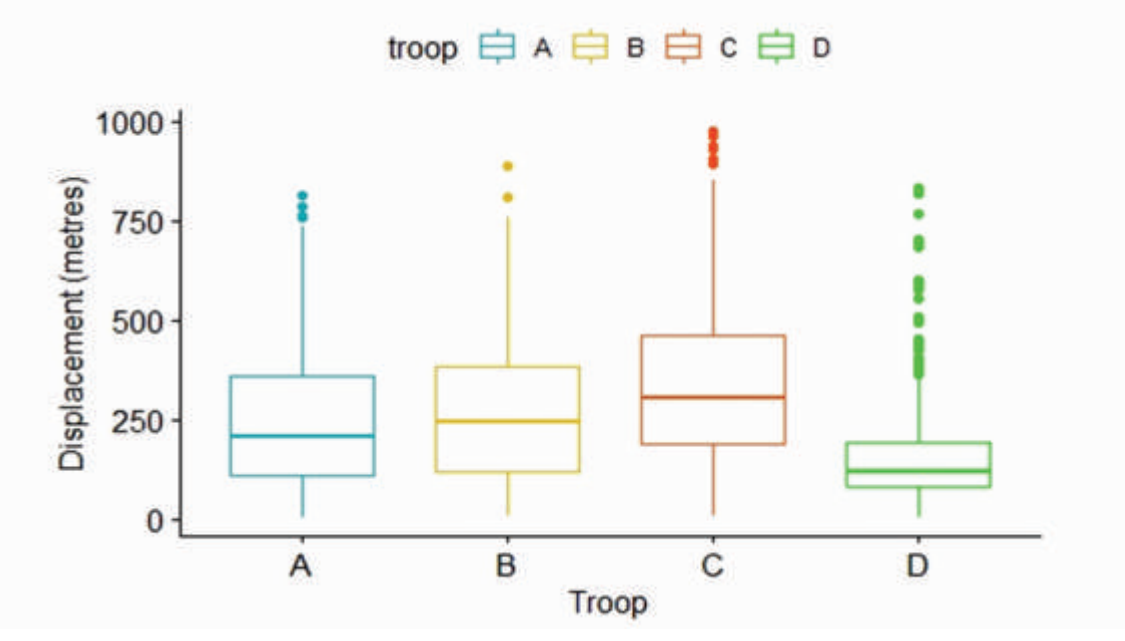


Figure 2.11 : *Troop wise data visualization for collared individual's daily displacement.*

Table 2.9 : Summarised daily displacements for the collared macaques.

Troop	Count (days)	Mean ± S.E. (metres)	Median (metres)
A	314	249 ± 9.82	210
B	219	273 ± 12.84	246
C	342	339 ± 11.41	303
D	249	167 ± 9.57	118

The adult female from Troop C recorded the highest mean daily displacement at 339 m (N=342) while Troop D had the lowest mean daily displacement at 167 m (N=249). Troops A and B recorded average daily displacements to be 249 m (N=314) and 273 m (N=219) respectively.

The mean ranks for the daily displacement for the four collared adult females showed significant differences to exist between them ($H=140.51$, $df=3$, $p < 0.001$). A pairwise comparison amongst the four collared individuals showed no difference between troops A and B ($p=0.204$). All other pairs were found to be significantly different from each other ($p<0.05$).

Habitat composition

A land use-land cover (LULC) map for the study area was created using Sentinel 2 data with five classes viz. agricultural fields, anthropogenically modified areas (human habitations, other structures etc.), roads, natural waterbodies, and vegetation (forests and plantations). Percentage area for each LULC class was calculated by overlaying the KDE LSCV home range contour on the map. While forests and allied natural vegetations comprised the maximum area, anthropogenic areas and roads were significantly high for both the troops.

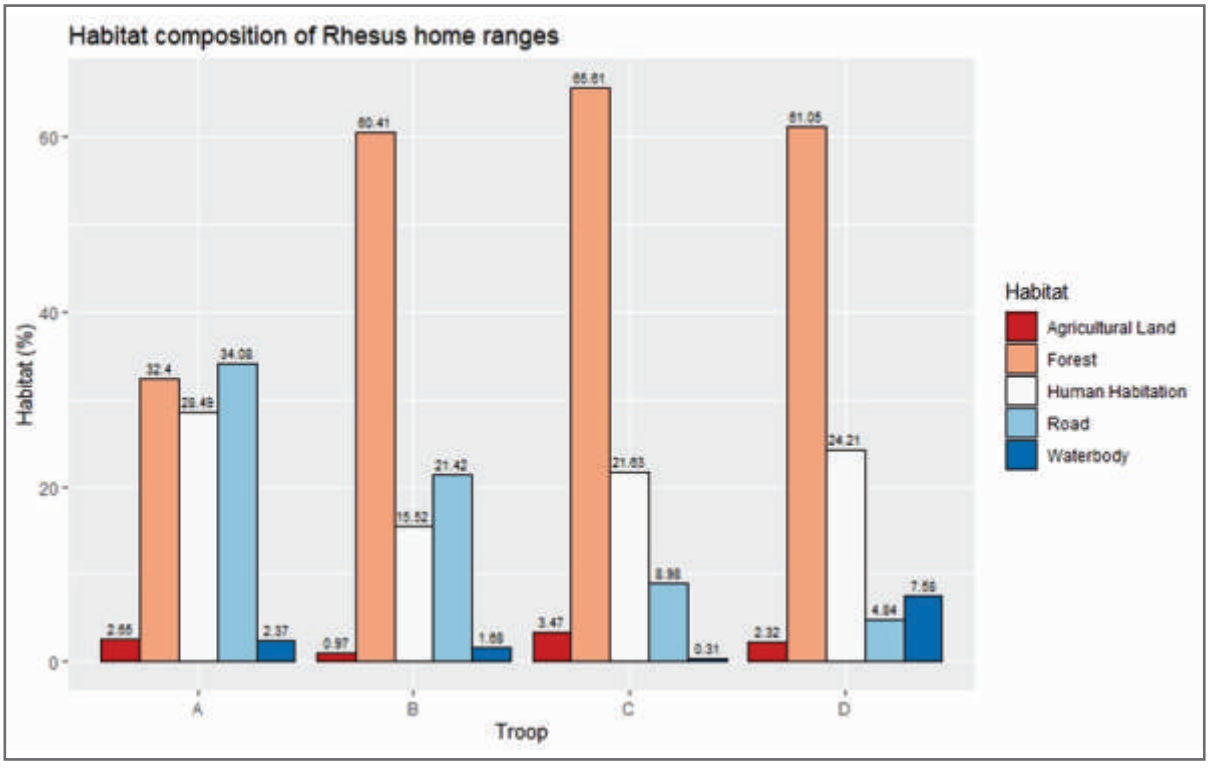


Figure 2.12 : *Habitat composition for the period 2018-2020; KDE LSCV home range.*

2.3.2 Updates for the period 2021-2022

One adult female from each of the identified and habituated socially distinct study troops, A and B, was collared with Ecotone GPS-GSM loggers programmed to take location fixes diurnally, every 15 minutes. Data was obtained for durations of the parturition (Summer) and pre-breeding seasons (Monsoon).

Home range and Core range sizes

The Kernel Density Estimation (KDE) and dynamic Brownian Bridge Movement Model (dBBMM) methods along with the Minimum Convex Polygon (MCP) were used to determine the home ranges (95%) and core ranges (50%) of the collared females. The reference bandwidth (href) and the least-squares cross-validation bandwidth (LSCV) were utilized as smoothing parameters for the analysis in case of KDE. Comparison with 100% MCP showed the KDE LSCV to be the best-fit predictor of home and core ranges for the study individuals. Both KDE href and dBBMM over predicted the home range sizes for the duration of the study.

Figure 2.13 : *Photographs for the two collared adult females, depicted as per their troops.*

Table 2.10 : Summarised details of deployed collars in study area around WII (2021-2022).

S. No.	Troop	Age-Sex Class	Associated Infant	Collar Id	Collar Frequency (MHz)	Date of Capture and Deployment	Data Obtained (days)	Parturition /Summer (days)	Pre-breeding/ Monsoon (days)
1.	A	Adult Female	Yes	MAKA10	164.14	19/04/2022	118	72	46
2.	B	Adult Female	Yes	MAKA03	163.07	30/05/2022	92	30	62

Table 2.11 : Home range (95%) and Core range (50%) sizes in hectare (1 ha=0.01 sq. km=10000 m^2).

Animal Id (Adult Female)	KDE LSCV 50% (ha)	KDE LSCV 95% (ha)	MCP 100% (ha)
B (MAKA03)	7.8	47	101
A (MAKA10)	4.5	26	84

The KDE LSCV volume contours showed significant overlaps in home and core ranges for both the troops within the Wildlife Institute of India campus. The area overlaps in the 100% MCP for both the troops was 61.81 ha. The home range and core range overlap were 18.64 ha and 1.21 ha respectively, for KDE LSCV.

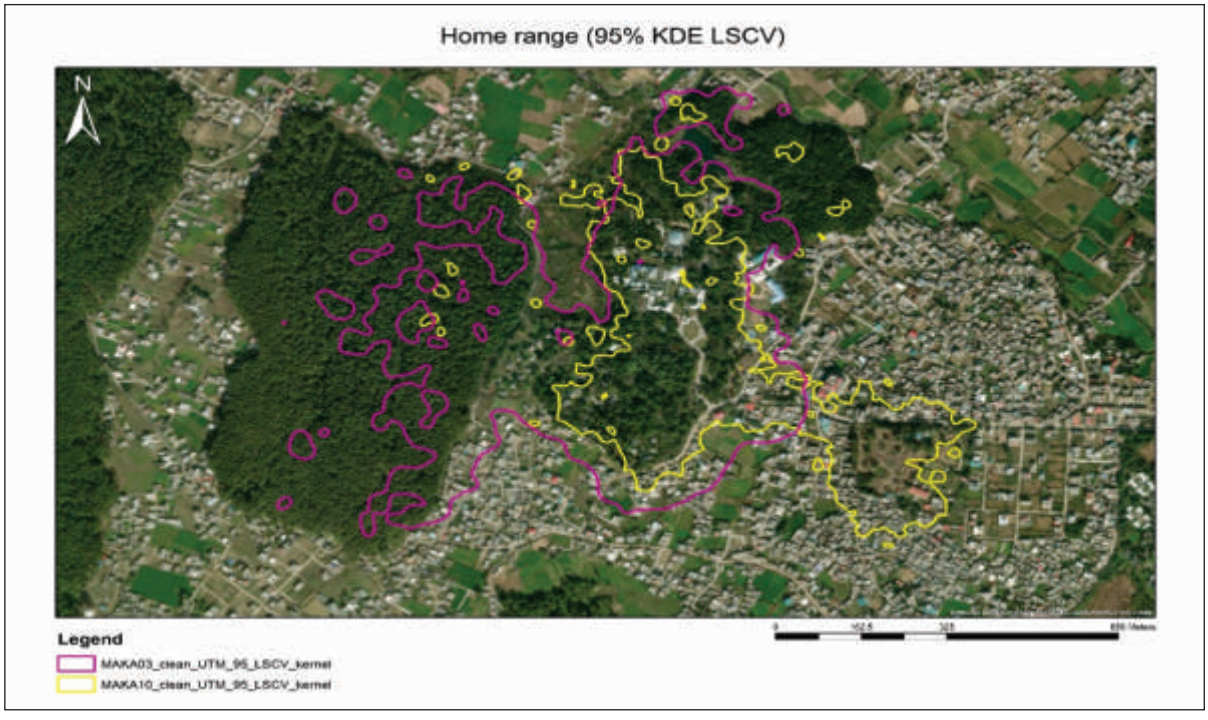


Figure 2.14 : *Home ranges (95% volume contours) for the collared adult females in the study area, LSCV bandwidth.*

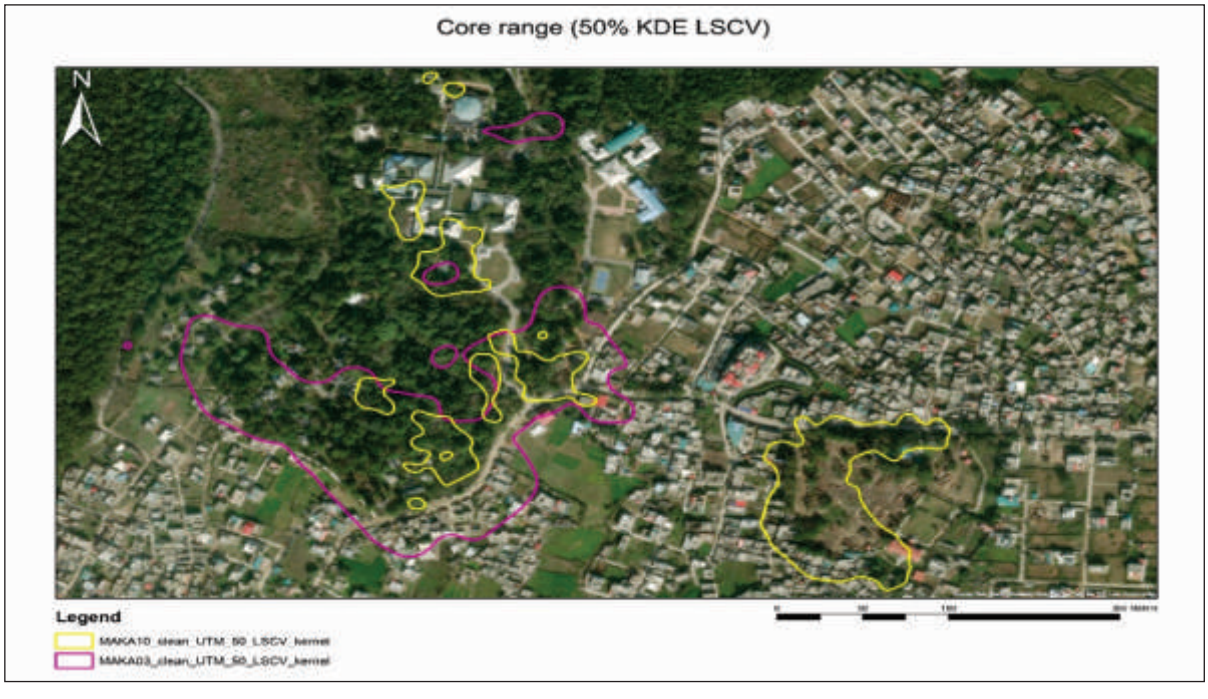


Figure 2.15 : *Core ranges (50% volume contours) for the collared adult females in the study area, LSCV bandwidth.*

Collared animal travel and movement

- Daily Travel Distance

Troop B exhibited a higher mean daily travel distance in comparison to Troop A.

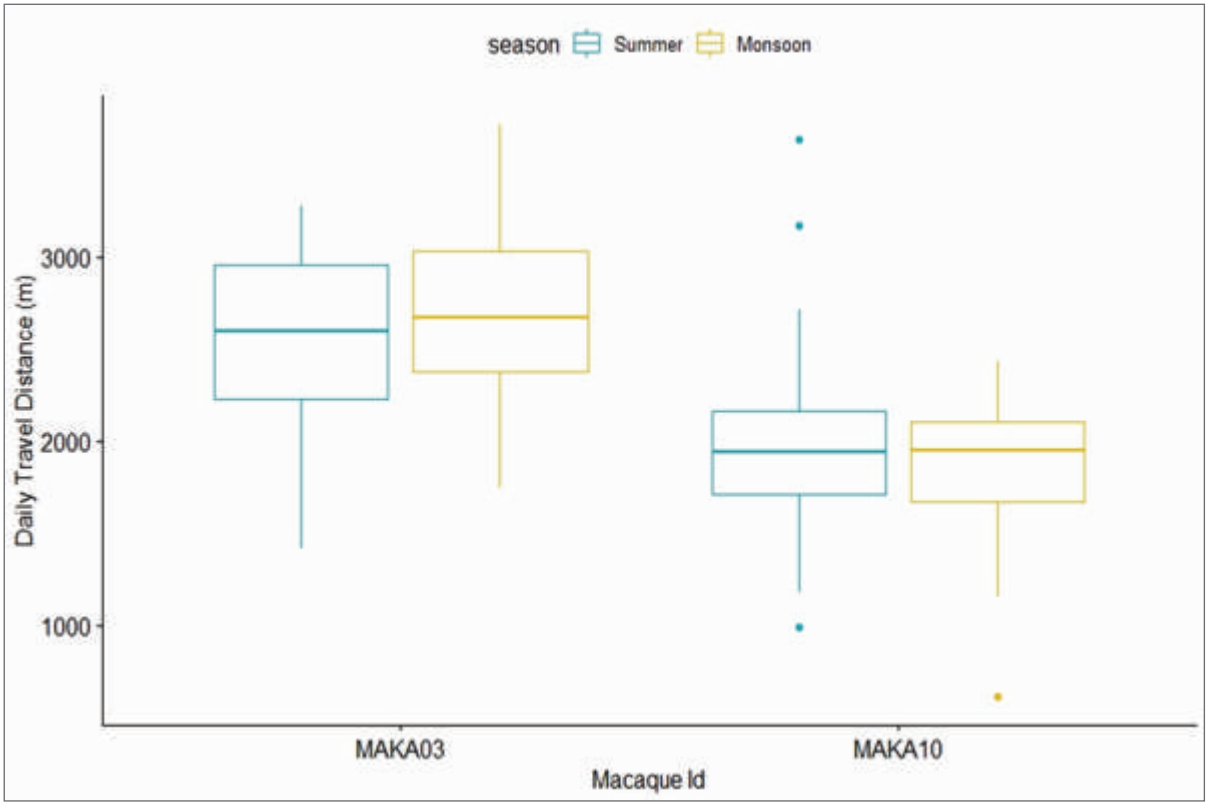


Figure 2.16 : *Troop wise and seasonal data visualization for collared individual's total daily movement.*

Table 2.12 : Summarised daily movement statistics for the collared macaques.

Animal Id	Season	Count (n)	Mean ± S.E. (km)
B (MAKA03)	Overall	92	2.62 ± 0.06
B	Summer	30	2.563 ± 0.09
B	Monsoon	62	2.69 ± 0.06
A (MAKA10)	Overall	118	1.92 ± 0.04
A	Summer	72	1.95 ± 0.05
A	Monsoon	46	1.87 ± 0.05

There was significant difference in the daily travel distances between the two study individuals (F=145.21, df=1, p=0.002) but no significant difference amongst the individuals with respect to the daily travel distance across seasons (F=0.021, df=1, p=0.886).

- Daily Displacement

The daily displacement for each collared rhesus macaque was obtained by calculating the straight-line distance between the first and the last geo-coordinate fixes for a day. The mean

daily displacement was then computed for both individuals.

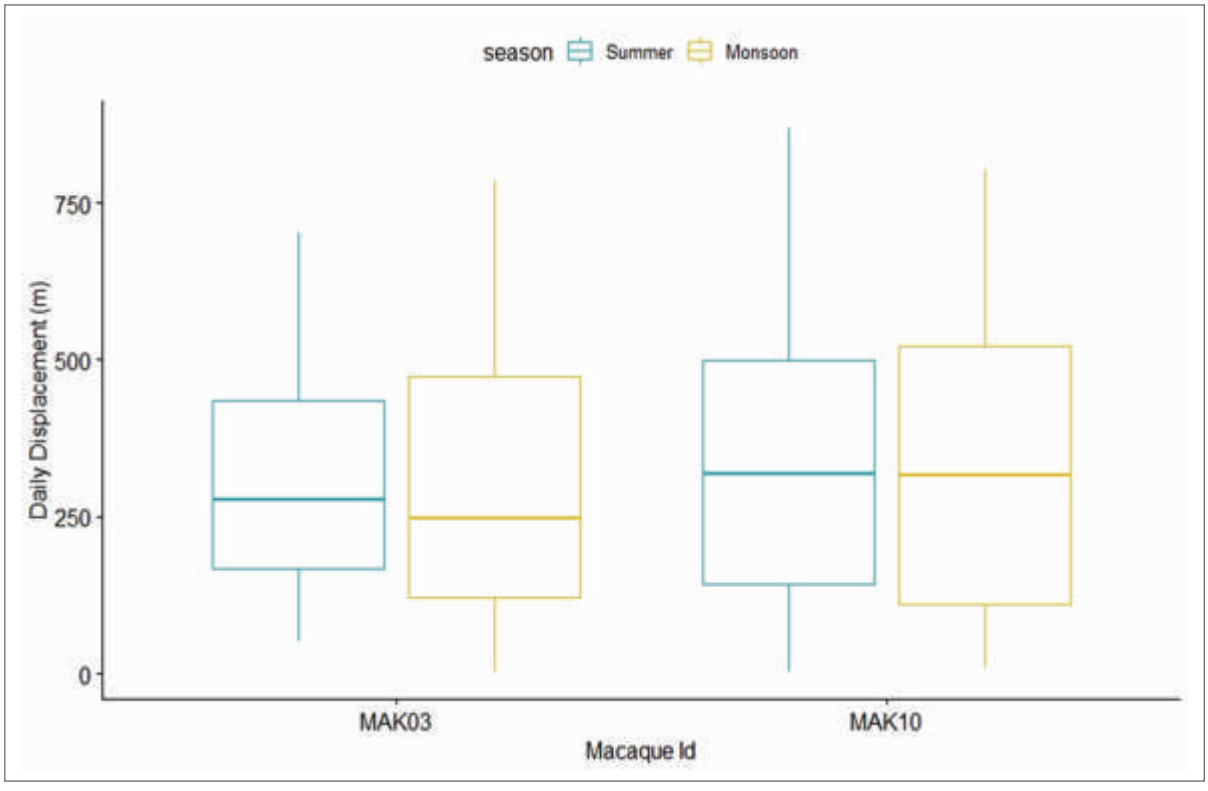


Figure 2.17 : *Troop wise and seasonal data visualization for collared individual's daily displacement.*

Table 2.13 : Summarised daily displacements for the collared macaques.

Animal Id	Season	Count (n)	Mean ± S.E. (m)
B (MAKA03)	Overall	92	304.32 ± 21.03
B	Summer	30	310.07 ± 31.43
B	Monsoon	62	301.54 ± 27.41
A (MAKA10)	Overall	118	327.04 ± 20.09
A	Summer	72	326.83 ± 24.7
A	Monsoon	46	327.36 ± 34.45

There was no significant difference in the daily displacements between individuals (F=0.595, df=1, p=0.441) and across seasons (F=0.012, df=1, p=0.915).

Habitat composition and selection ratios

A land use-land cover (LULC) map for the study area was created using Sentinel 2 data with five classes viz. agricultural fields, anthropogenically modified areas (human habitations, other structures etc.), roads, natural waterbodies and vegetation (forests and plantations). Percentage area for each LULC class was calculated by overlaying the home range contours on the map. While vegetation comprised the maximum area, anthropogenic areas and roads were significantly high for both the troops.

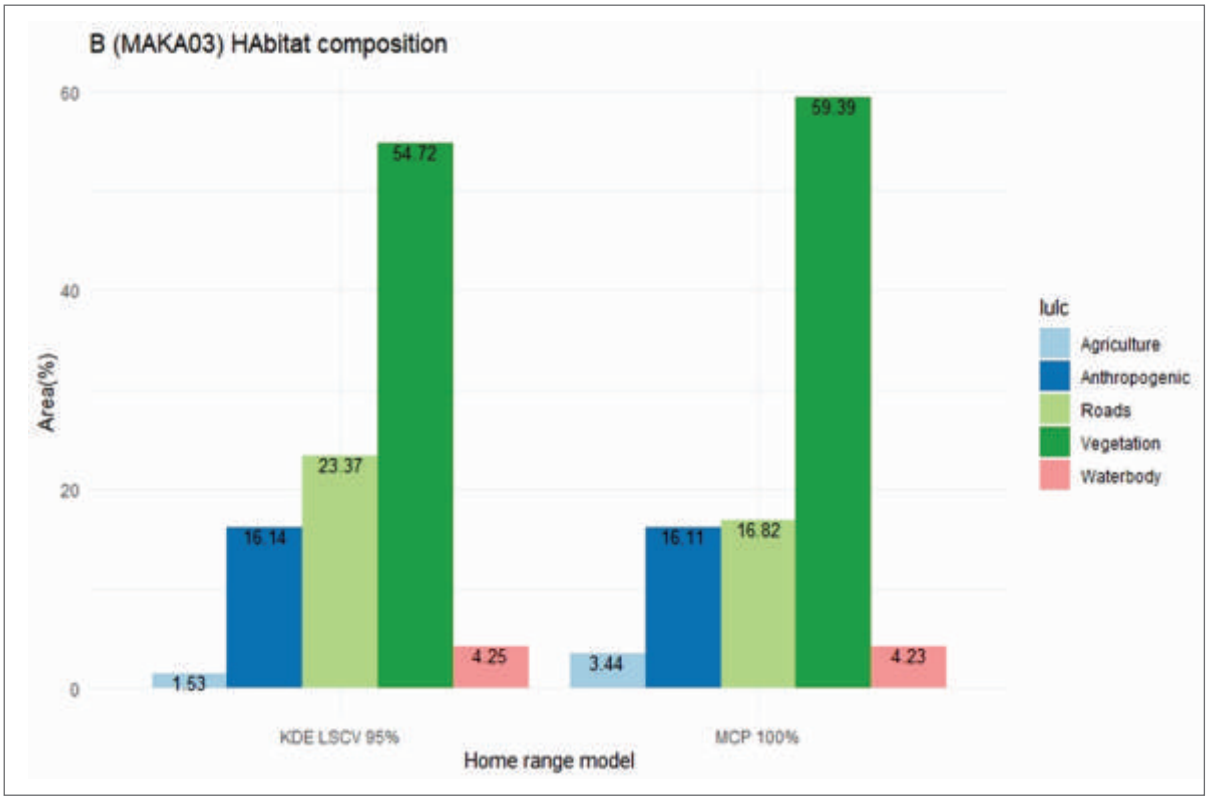


Figure 2.18 : *Habitat composition for Troop B (MAKA03).*

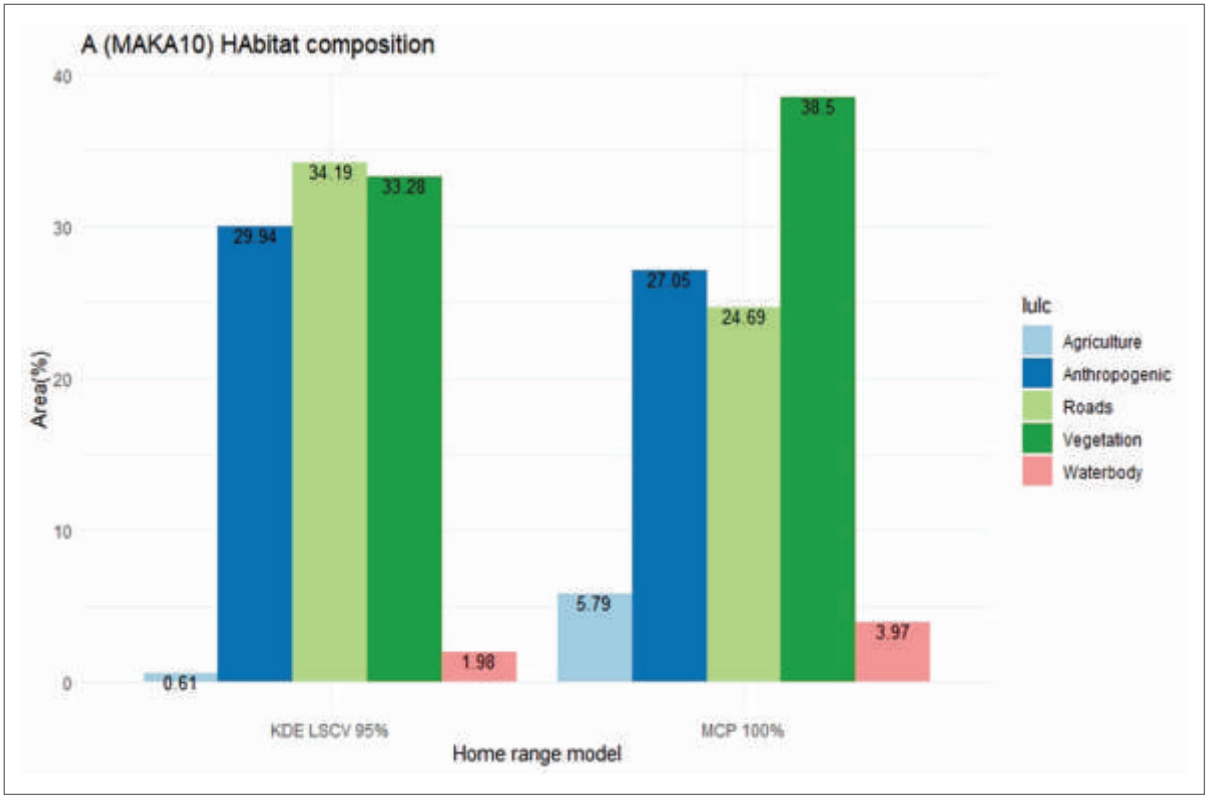


Figure 2.19 : *Habitat composition for Troop A (MAKA10).*

Manly's habitat selection ratios were calculated using a Design III study whereby, both the collared individuals were using habitats differently within the study area (Manly et al. 2002). Habitat class areas from 100% MCP were used as available while 95% KDE LSCV habitat classes were designated as utilized. Habitat selection for each animal was significantly different (Log-likelihood; df=4; $p<0.05$). The overall habitat selection was also statistically significant (Log-likelihood; df=8; $p<0.05$). Both global and individual habitat selection ratios showed roadsides and anthropogenic built-up areas to be used more than their availability. Though crop raiding is observed, agricultural fields and crop lands were used less than available due to reduced residence times and active deterrence in these areas. Proximity to anthropogenic food sources

(garbage dumps and provisioning) and ease of access to vegetation-anthropogenic habitat edges as refuge sites could be an important factor for the habitat selection patterns observed.

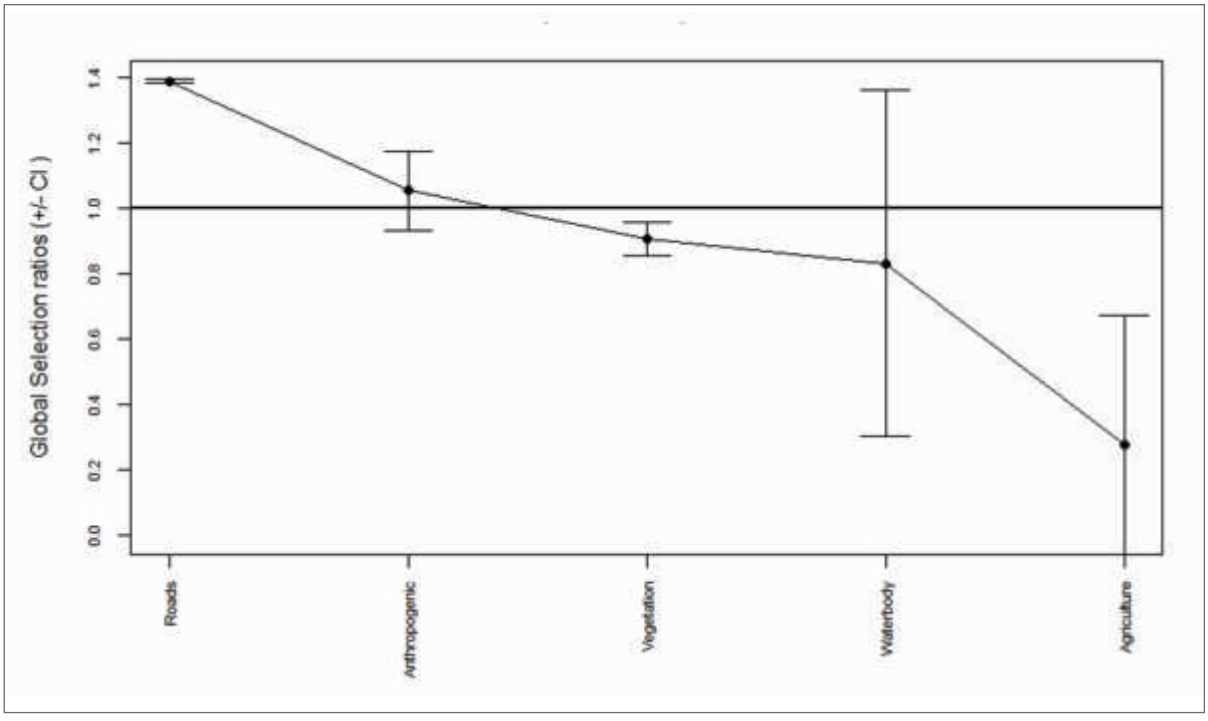


Figure 2.20 : *Global Selection Ratios, Manly's Design III study.*

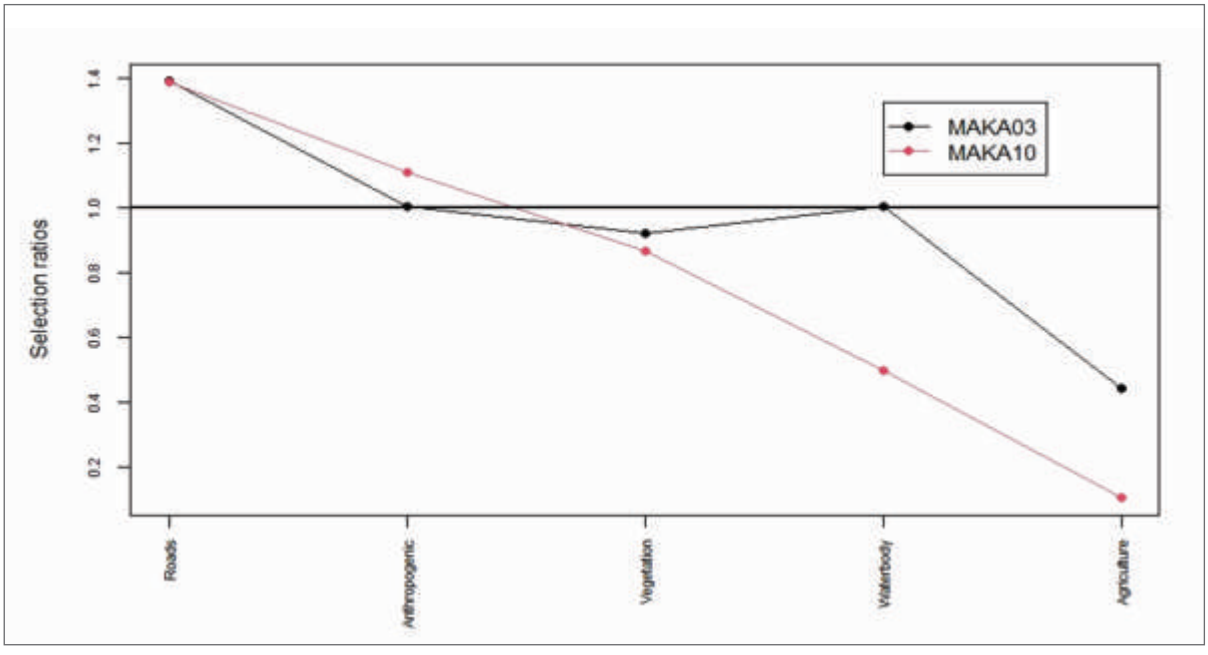


Figure 2.21 : *Individual Selection Ratios, Manly's Design III study.*

Chapter 3

BEHAVIOURAL MONITORING AND OBSERVATIONS IN RHESUS MACAQUES

Uddalak Tathagato Bindhani, Mariyam Nasir, Deepika Boora, Sanath Krishna Muliya, Sarvesh Kumar, Supravat Mahata, Divya Dwivedi, Chandrapratap Singh Chandel, Yati Gairola, Vishnupriya Kolipakam, Lallianpuii Kawlni, YV Jhala, Qamar Qureshi

With troops often comprising a large number of individuals, the rhesus macaque is an actively social animal. While large troops are usually found to inhabit urban landscapes, smaller troops tend to be encountered more frequently in natural habitats such as forests. The ecological adaptability and behavioural flexibility of the rhesus, has allowed them to inhabit a wide range of habitats while utilizing a diverse array of resources. A rhesus macaque troop comprises of large multi-male, multi-female associations and characterized with female philopatry, with the female individuals forming dominance hierarchies according to matrilineal kinship. Understanding the behaviour of the species is thus crucial to quell conflict scenarios, in a successful management strategy.

Behavioural sampling of each identified and habituated troop is conducted once every two weeks throughout the year, across seasons. The targeted study troop is located and behavioural sampling is initiated early in the morning (5:30 AM-6:30AM) with the animals being followed throughout the day into late evening, when visibility is lowered. Alternate scan and focal sampling of 10-minutes duration each is undertaken throughout the day with an interval of 5 minutes across each record to capture variation in troop behaviour and compensate for observer fatigue. The scan sampling is used to record the instantaneous

activities of all visible members of the troop. The focal sampling is used to record all behaviours of a randomly selected visible individual for 10 minutes (Altman 1974; Bateson and Martin 2021). Four age-sex classes have been designated for the rhesus macaques; adult males and adult females (sexually mature with prominent visible secondary sexual characteristics), juveniles (sexually immature with absence of developed secondary sexual features), and infants (unweaned minors dependant on mother). An ethogram defining all behaviours of interest has been prepared to facilitate observation and record of behaviours in the field.

Table 3.1 : Ethogram for Rhesus macaques with behaviours of interest.

Behavioural States	Description (Behavioural Events)
Stationary/resting	Lying on its back or sideways, sitting.
Aggression	Aggressive behaviours not listed individually – can include: biting, hitting, grabbing, charging, chasing, bare canines.
Movement	Walking, running, jumping, locomotion.
Feeding	Eating, ingesting into cheek pouch, search/forage.
Grooming	Autogrooming, allogrooming.
Sexual behaviour	Elicitation, mounting, mating, checking anogenital region, nipping, nibbling, checking vaginal region.
Playing	Playing behaviours not limited to climbing, swinging, play-fighting, mock charging.
Out of sight	Animal has moved out of sight and cannot be seen.

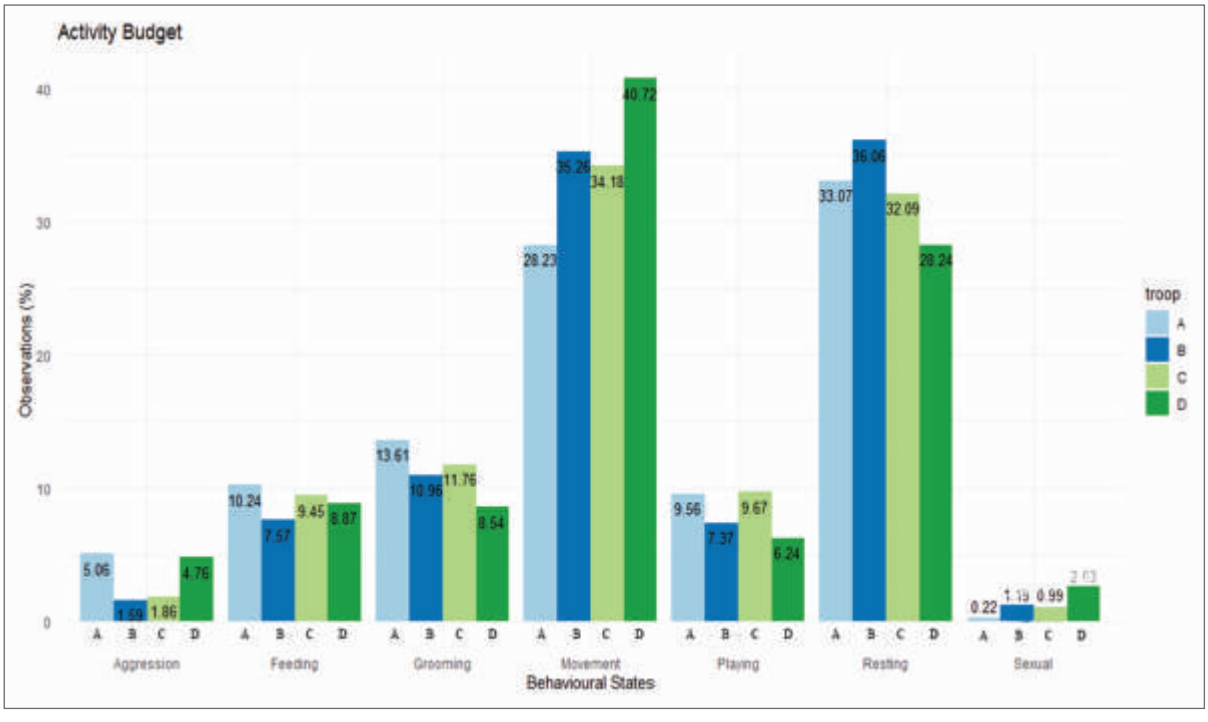


Figure 3.1 : *Activity budget for all four Rhesus troops obtained from behavioural scan sampling.*

The activity for each behavioural state was calculated as the percentage of the frequency score obtained during scan observations within each troop across age-sex classes (Bateson and Martin 2021). Maximum activity was observed in the behavioural states of 'movement' and

'resting' for all four troops, followed by 'grooming' and 'feeding'. Sexual activity being seasonal (October-January) had the lowest frequency for all four troops. Frequency scores for 'playing' were mostly obtained from the juveniles and infants in a troop, with adults rarely participating in play behaviour.

3.1. Feeding habits

While natural food resources include perennial and seasonal plant parts (leaves, fruits, flowers, bark, sap etc.) and insects, anthropogenic food resources are obtained via feeding at garbage dump sites, raiding homes and cultivations, and via active provisioning by humans.

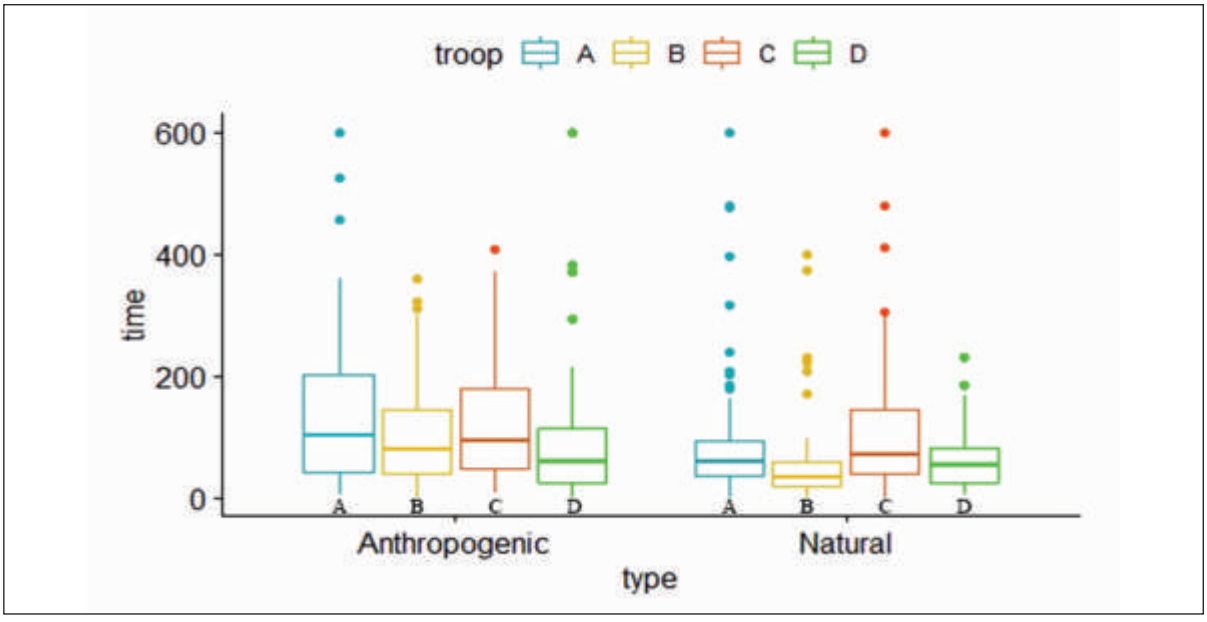


Figure 3.2 : *Data visualization for time spent by the four troops on the two food resource types.*

Table 3.2 : Summarised statistics for time spent on the food resource types.

Troop	Food Type	Count (N)	Mean ± S.E. (seconds)
A	Anthropogenic	27	161 ± 30.98
A	Natural	95	88.2 ± 10.45
B	Anthropogenic	59	108 ± 12.17
B	Natural	58	59.2 ± 10.57
C	Anthropogenic	56	128 ± 13.89
C	Natural	61	118 ± 16.13
D	Anthropogenic	37	100 ± 20.88
D	Natural	26	67.3 ± 11.08

The average time spent feeding on anthropogenic food resources was higher for all four troops in comparison to natural food resources (Figure 3.2). Even if the observations for natural food were higher for certain troops, the mean time spent was lower in comparison to anthropogenic resources. The four study troops exhibited significant difference with respect to time spent on the two food types viz. natural and anthropogenic food resources (F=9.56, df=1, p=0.002). All

four study troops showed similar trends in the time invested on the two food resource types and no significant difference was observed amongst the troops (F=2.06, df=3, p=0.104). The higher time spent on anthropogenic food resources could be alluded to its clumped distribution, search and handling times, and higher energy returns.

3.2 Grooming habits

The evolution of grooming behaviour amongst social primates has been attributed as a functional trait to reduce stress and antagonism amongst individuals while promoting the formation and strengthening of social bonds (Strier 2015). Synanthropic primate species experience further stress due to agonistic interactions with humans and higher intra-troop competition for clumped resources. Grooming also allows for keeping ectoparasite infestation and transmission levels at a check due to aggregation of a large number of individuals in anthropogenic landscapes.

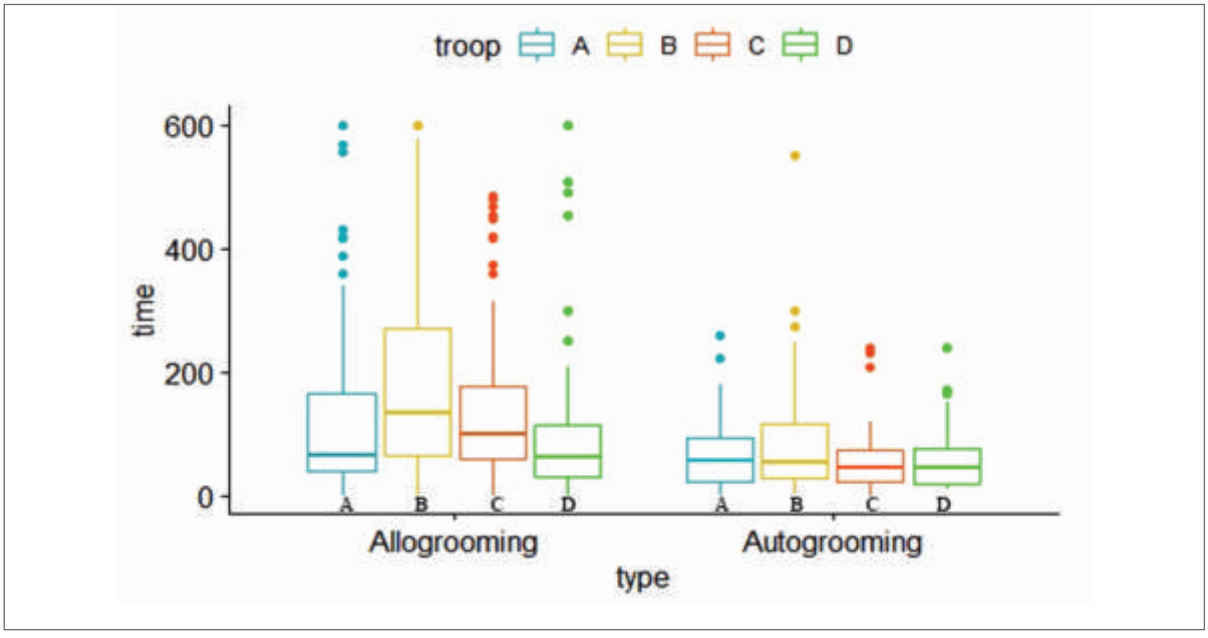


Figure 3.3 : *Data visualization for time spent grooming by the four troops.*

Table 3.3 : Summarised statistics for time spent on the food resource types.

Troop	Grooming (type)	Count (N)	Mean ± S.E. (seconds)
A	Allogrooming	115	126 ± 13.05
A	Autogrooming	66	64.6 ± 6.54
B	Allogrooming	68	184 ± 19.52
B	Autogrooming	47	85.2 ± 14.44
C	Allogrooming	103	137 ± 11.63
C	Autogrooming	37	61.1 ± 9.67
D	Allogrooming	72	105 ± 15.44
D	Autogrooming	33	60.2 ± 9.40

The average time spent allogrooming was higher for all four troops in comparison to autogrooming. There was significant difference in time spent on the two grooming types by the four troops ($F=11.43$, $df=1$, $p<0.001$) and also amongst the troops with respect to time being spent on the grooming types ($F=5.64$, $df=3$, $p=<0.001$). A pairwise comparison across troops showed significant differences for grooming time to exist between troops A–B ($p=0.025$) and between troops B–D ($p=0.005$).

3.3 Individual identification, dominance hierarchy and social networking in Rhesus macaques

3.3.1. Dominance Hierarchy

Rhesus macaques are highly social primates living in complex societies characterized by large multimale-multifemale troops, female philopatry and male dispersal. Females remain in their natal groups and establish dominance hierarchies based on matrilineal kinship, while males, upon attaining sexual maturity, leave their natal groups at the beginning of the breeding season and compete for mating opportunities with females from other groups (Melnick et al. 1984). Dominance in rhesus macaque societies is of high ecological value directly influencing evolutionary fitness, as high-ranking individuals have priority access to food, mates, and space (Lindburg 1971).

High-ranking female Rhesus macaques have greater access to feeding sites and are less likely to be challenged during feeding compared to lower-ranking females. However, low-ranking females can still consume the same amount of food by storing it in their cheek pouches and moving away from the group to eat, although this method of feeding is more energetically expensive (Deutsch and Lee 1991).

Female dominance rank is stable over a lifetime and is passed down to their female offspring. In contrast, male dominance rank is not stable and is determined by a combination of social interactions and aggressive skills (Lindburg 1971; Seth 2000). Agonistic interactions and submissive gestures are used to establish and reinforce social position in Rhesus macaques in both sexes with males being usually dominant over females (Lindburg 1971; Berard 1999).

Dominance hierarchy is important for the social stability of macaque troops. Understanding social hierarchy and identifying individuals within troops is imperative in implementing targeted wildlife management practices aligned with the biology of the species (viz. reproductive control, surgical sterilization etc.), to alleviate localized human-macaque conflict.

Social hierarchies are a result of agonistic dyadic interactions between troop members where there is a winner (dominant individual) and a loser (subordinate individual) (Drews 1993). The prevalence and importance of social hierarchies in nature has led to the development of indices for inferring dominance hierarchies based on observed social dyadic agonistic interactions. Index ratings are calculated based on success or ratings from which individuals can be further ranked as per requirement. Social hierarchy indices also allow the advantage to be subjected to

statistical tests and tests of linearity. Indices are calculated either from a temporal sequence of interactions of identified individuals (Elo-rating; Elo 1978) or from an interaction matrix of the participating individuals (David's score; David 1987).

3.3.2 Social Networking

Social network analysis (SNA) is the study of social structures, relationships, and interactions among individuals, groups, organizations, or even entire societies. It can be used to analyse various aspects of social life, information flow, social influence, and power dynamics (Newman & Park, 2003).

In group-living species, individuals gain significant advantages from establishing an extensive network of social relationships. This results in complex organizations that are difficult to quantify in a comprehensive manner. In this respect, network analyses are an ideal means to pinpoint the overall properties of social structures, and the place of each individual within these structures (Proulx et al., 2005).

Animal social network analysis is a field of study that focuses on the patterns of social interactions and relationships within animal groups. It involves the use of mathematical and computational tools to analyse the social networks of animals, including their patterns of communication, cooperation, competition, and social structure –(Wey et al., 2008). Some of the key applications of animal social network analysis include identifying key individuals within a social group, predicting the spread of disease within a population, and understanding the impact of environmental factors on social behaviour to further assist in conservation (Croft et al., 2008).

Social network analysis can be used to examine the structure of non-human primate social networks, including the patterns of social interactions, the role of individual primates within the network, and the factors that influence social organization. SNA can be used to study a range of behaviours, including grooming, play, aggression, and cooperation (Sueur et al., 2011).

Social networks tend to be structured around a small number of individuals who play a critical role in maintaining social cohesion. These key individuals have a large number of social connections and play a crucial role in transmitting information, maintaining social bonds, and facilitating social learning (Funkhouser et al., 2018). Further, social network analysis can help identify individuals who asymmetrically participate in aggression and guide management in modifying group composition (e.g., remove/introduce individuals, divide large subgroups if deemed appropriate) to achieve network stability (Balasubramaniam et al., 2018, 2019).

Overall, social network analysis has provided valuable insights into the complex social behaviour of non-human primates, and has helped to better understand the factors that shape primate social networks and influence social behaviour.

There are a set of properties exhibited by a social network which can be used for analysing various social components of any network (Wasserman et al., 1994). Some of these properties

relevant to the current study are explained as follows:

Nodes: The individuals or entities in the network are called nodes. In SNA, nodes can be people, organizations, or any other unit of analysis.

Edges: The connections between the nodes are called edges. These edges can be directed (one-way) or undirected (two-way) and can be weighted or unweighted, depending on the type of data being analysed.

Centrality: The centrality of a node refers to its position in the network. Centrality measures include degree centrality, betweenness centrality, pagerank centrality and eigenvector centrality.

Clustering: Clustering refers to the tendency of nodes to form clusters or groups within the network. Clustering coefficient measures how tightly connected a node's neighbours are.

Path length: Path length is the number of edges it takes to get from one node to another. Shortest path algorithms can be used to find the shortest path between two nodes in a network.

Methods

Data was collected in the habituated study troop B, ranging across anthropogenically modified habitats (human habitation, croplands, roads, garbage dump sites etc.) and fragmented forest

(sal plantations and mixed forests) patches. The troop comprised of ~65 individuals overall across four age-sex classes (adult males, adult females, juveniles, and infants).

A preliminary reconnaissance survey was conducted whereby all adults within the troop were observed, photographed, and catalogued. Natural markings, facial features and physical characteristics were documented for each adult individual and a relevant name assigned for ease of recognition in the field. A total of 19 adult females and 8 adult males were individually identified and observed during the exercise.

Table 3.4 : Individual identities of adult individuals in study troop B.

S. No.	Individuals	Age-Sex Class	Acronym used for Networking
1	Red face	AF	RF
2	Ape face	AF	AF
3	Cleft	AF	Cl
4	Sister Cleft	AF	SC
5	White eye	AF	WE
6	Unibrow	AF	Un
7	Tongue	AF	To
8	Red HF	AF	RHF
9	White HF	AF	WHF
10	Long lash	AF	LL
11	Monroe	AF	Mon
12	Molly	AF	Mo
13	Polka	AF	Po
14	Ear tag	AF	ET
15	White collar	AF	WC
16	White tape	AF	WT
17	Black collar	AF	BC
18	Socket	AF	So
19	Sally	AF	Sa
20	Beardy	AM	Be
21	Matku	AM	Ma
22	Blue collar	AM	BuC
23	Thakur	AM	Th
24	Peripheral	AM	Pe
25	Wart	AM	Wa
26	Van Gogh	AM	VG
27	Kaptan	AM	Ka

The troop was followed and interactions sampled for a minimum of 8 contact hours per day using ad-libitum behavioural sampling technique to record dyadic agonistic interactions between adult members of the troop (Altman 1974; Bateson and Martin 2021). Interactions were documented for a total of 320 contact hours from July 2022 to January 2023, across the monsoon and winter seasons of the study area.

An ethogram for agonistic and affiliative behaviours was created via observations during the initial survey and from prior published literature on macaque behaviour. Agonistic behaviour was further classified into aggressive and submissive behaviours, as follows:

Table 3.5 : Ethogram of agonistic and affiliative behavioural events sampled.

Eyebrow raise	Agonistic	Aggressive
Displacement	Agonistic	Aggressive
Facial threat	Agonistic	Aggressive
Hand threat	Agonistic	Aggressive
Chase	Agonistic	Aggressive
Push	Agonistic	Aggressive
Pull	Agonistic	Aggressive
Mount	Agonistic	Aggressive
Bite	Agonistic	Aggressive
Grimace	Agonistic	Submissive
Allogroom	Affiliative	
Allogesture	Affiliative	
Anogenital presentation	Affiliative	
Follow	Affiliative	
Mating	Affiliative	

For each interaction observed during a sampling session, the time, participants, kind of behaviour exhibited and the winner-loser of the event was recorded in sequence.

Dominance hierarchies were determined based on the David's score and the randomized Elo-rating indices (Sanchez-Tojar et al. 2017). All analyses were undertaken using the statistical software R using the aniDom (Sanchez-Tojar et al. 2017), socialh (Valente et al. 2022) and Elorating (Neumann and Kulik 2020) packages. The David's score is calculated by reorganizing the interaction matrix to calculate the numerical criterion, by maximizing or minimizing for the whole matrix, as a suitable measure for individual overall success (Gammell et al. 2003). Randomized Elo-rating reduces the effects of temporal biases and negates errors during data collection based on the sequential method while allowing the calculation of uncertainty in overall ranks of participating subjects (Sanchez-Tojar et al. 2017). Analysis was done for two sets viz. all adult individuals (males and females combined) and the adult females separately.

For creating social networks, individual ids were used as nodes and the frequencies of interactions were the edges of network. Dyadic interactions obtained via ad-libitum sampling were used to create adjacency matrix, for both affiliative and agonistic interactions in R, using package igraph (Csardi & Nepusz, 2006). Adjacency matrix is a squared matrix with individual identities as row and columns, and the frequency of interaction as the matrix cell value. By default, the direction of any interaction is from row individuals to column individuals. Using this matrix, weighted-directed social networks were created for affiliative and agonistic interactions. Further, network properties like Eigenvector Centrality and PageRank Centrality were computed for affiliative network to measure the level of influence of an individual within

a troop –(Das et al., 2018; Newman, 2005). Each individual within the network was given a score or value, the higher the score the, greater the level of influence within the troop. For agonistic network the degree centrality (in-degree and out-degree) was calculated to identify individuals who participate in agonistic behaviour more frequently than others in a troop.

3.3.3 Results and Discussions

- Dominance Hierarchy (Adult females and adult males combined)

Randomized Elo-rating showed the dominant males to be ranked above females as is observed in macaque societies. Subordinate males were observed to be intermediate rankers amidst the females. Estimates of uncertainty by repeatability and uncertainty by splitting were 0.87 and 0.67 respectively, indicating a stable and robust hierarchy with low uncertainty in randomized interactions.

Ranks based on David's scores and normalized David's scores predicted a slightly different pattern for the subordinate males. David's scores varied between 111.75 to -84.75 while the normalized David's scores ranged from 17.14-9.86. The steepness based on normalized David's score was 0.21 and statistically significant (p = 0.001). The test for dominance linearity indicated a significant linearity in the hierarchy of all adults (h' = 0.32, p = 0.001). The Landau index also predicted a strong linearity based on the agonistic dyadic interactions (h = 0.86). The sociometric matrix for all adults of troop B is depicted in, where the animals have been mentioned around a circular plot with the thickness of the lines are directly proportional to the frequency of interactions between individuals while the arrows indicate directionality (Figure 3.5).

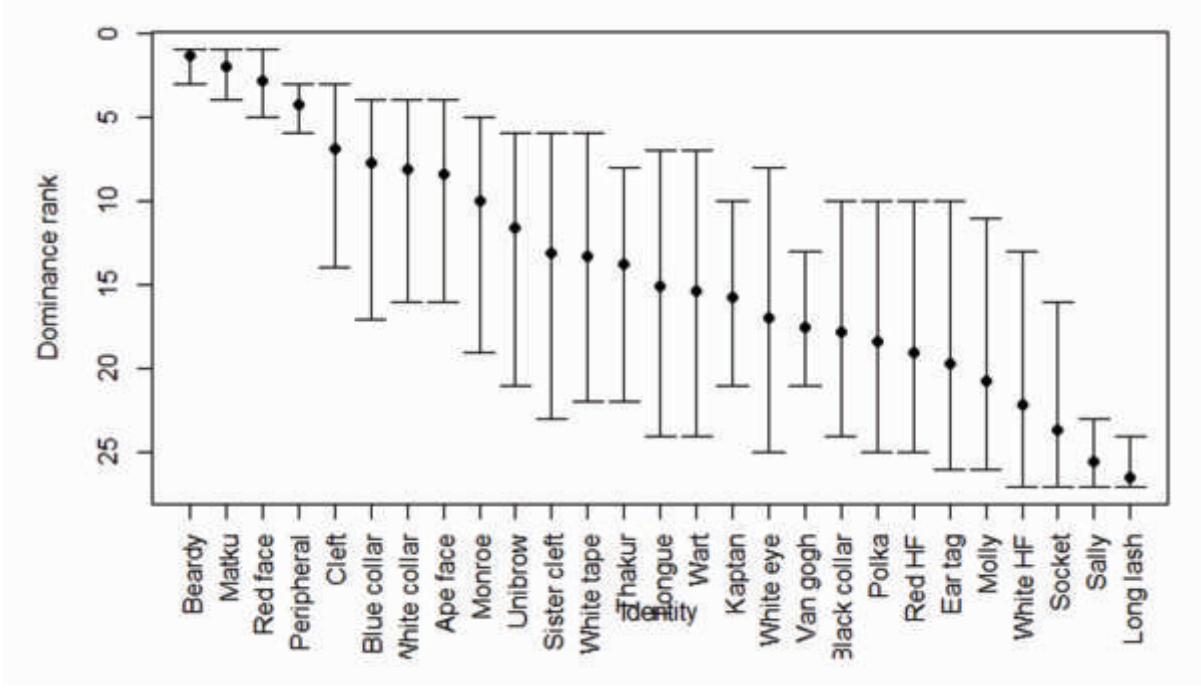


Fig 3.4: *Randomized Elo-rating dominance ranks with 95% CI for adults in study troop B.*

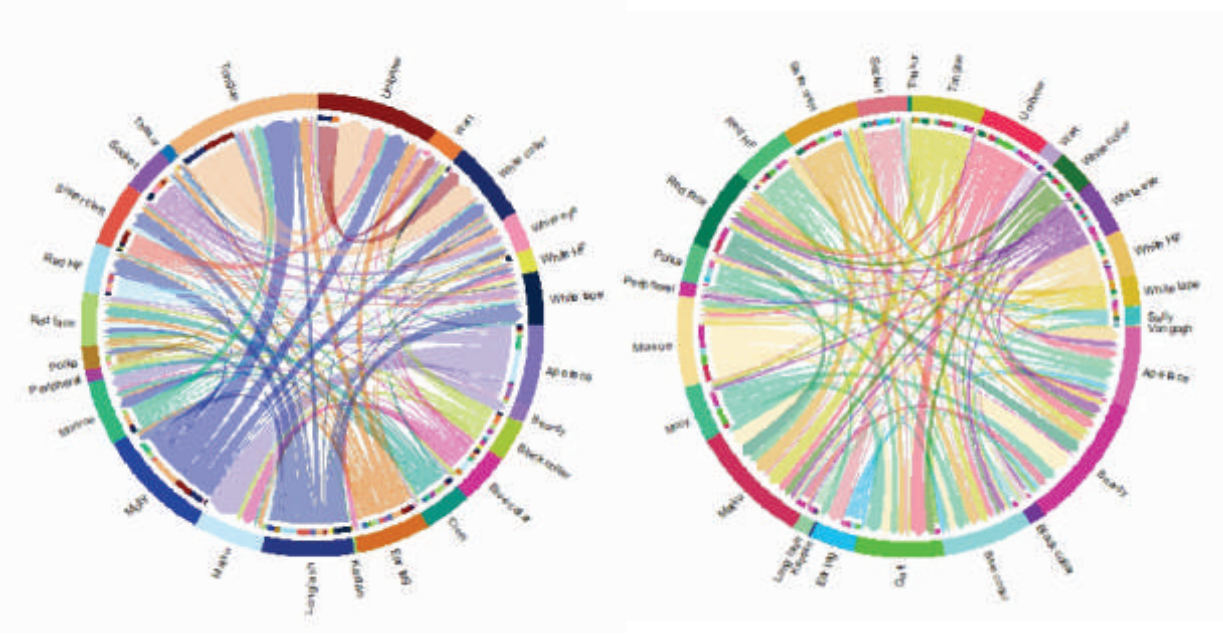


Figure 3.5 : *Winner and loser sociograms based on dyadic interaction frequencies of all adults (male and female).*

- Dominance Hierarchy (Adult females)

Randomized Elo-rating clearly differentiated the high-ranked and low-ranked individuals with intermediate individuals in between showing a larger confidence interval for individual ratings. Estimates of uncertainty by repeatability and uncertainty by splitting were 0.87 and 0.74 respectively, indicating a stable and robust hierarchy with low uncertainty in randomized interactions.

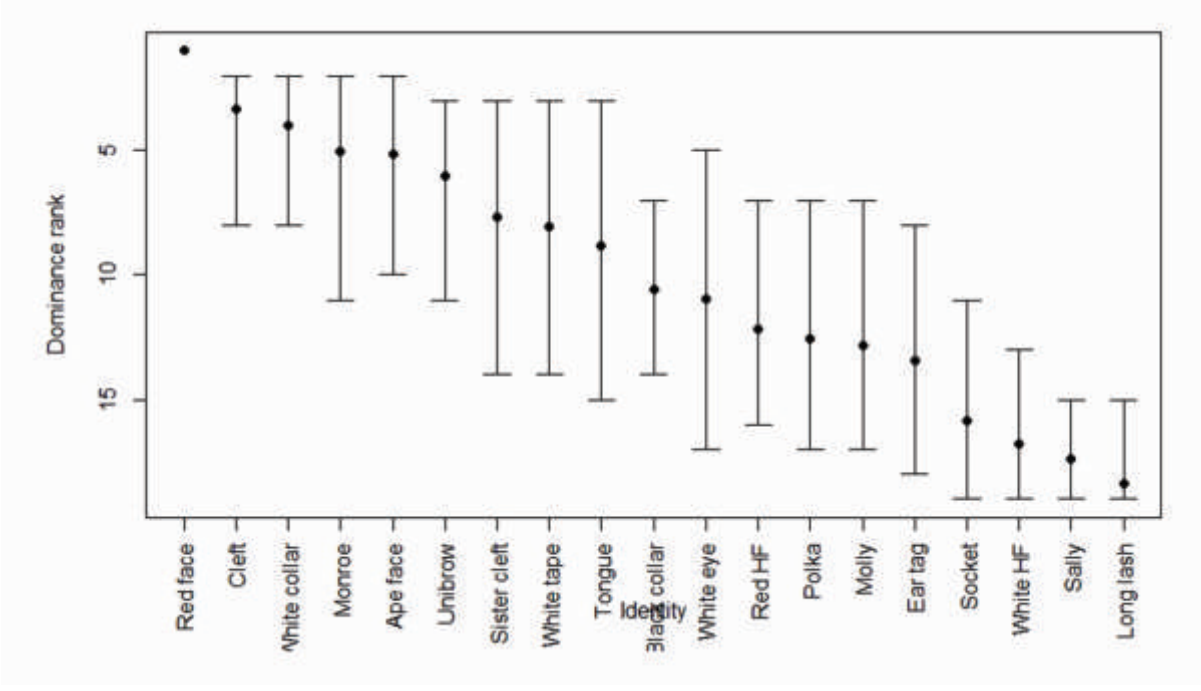


Fig 3.6: *Randomized Elo-rating dominance ranks with 95% CI for adult females in troop B.*

Ranks based on David's scores and normalized David's scores predicted a slightly different pattern for the intermediate females. David's scores varied between 77.6 to -58.81 while the normalized David's scores ranged from 13.08-5.9. The steepness based on normalized David's score was 0.29 and statistically significant (p = 0.001). The test for dominance linearity indicated a significant linearity in the hierarchy of all adult females (h' = 0.43, p = 0.001). The Landau index also predicted a strong linearity based on the agonistic dyadic interactions (h = 0.76). The sociometric matrix for all adult females of troop B is depicted in Figure 3.7.

The individual identities and social hierarchy of individuals could be used to track intra-troop dynamics, mating opportunities and reproductive behaviour. This shall allow informed decisions to be made for ethical and efficient management practices in alleviating conflict scenarios and promoting long-term human-macaque coexistence. A priori understanding of a focal troop's social dynamics allows for intervention measures to be planned and implemented reducing negative impacts on the species.

- Social Networking

Affiliative Network

Figure 3.8 and 3.9 represents the social network for affiliative interactions. The nodes represent individual identities of adult male and adult females of the troop. Nodes are connected by edges, here edges represent the affiliative interaction, and the width of these edges describe the intensity of these interactions. The arrows represent direction of affiliative interaction, which can be unidirectional or bidirectional. In Figure 3.9 the width of nodes represents degree centrality of adult macaques. The bigger the size of node, the more connected the individual is to other members of the troop. Eigenvector centrality and

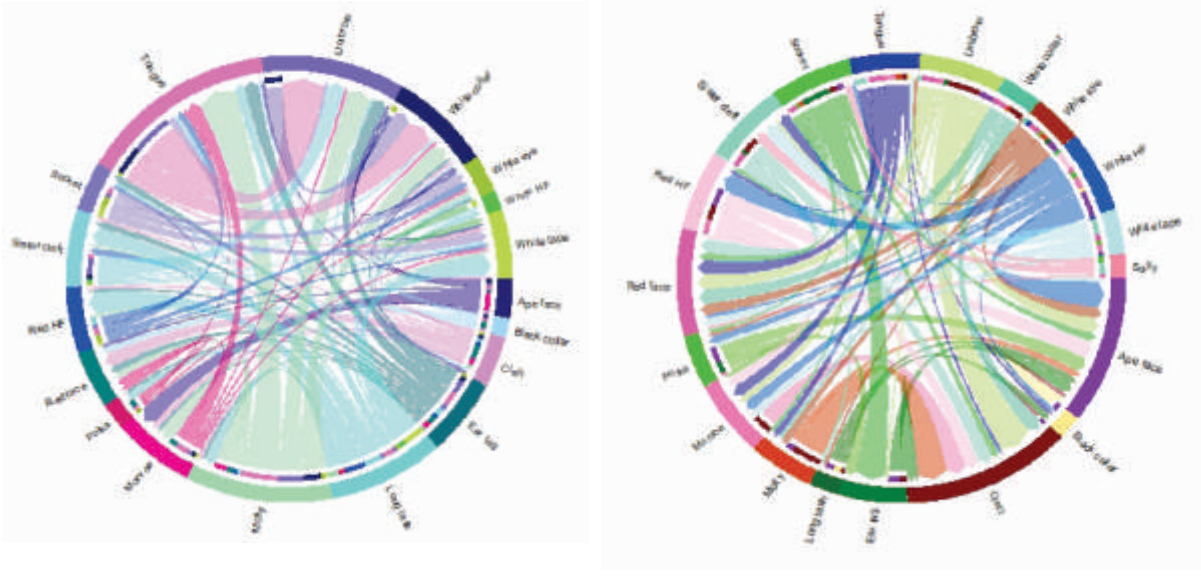


Fig 3.7: *Winner and loser sociograms based on dyadic interaction frequencies of adult females.*

PageRank centrality values for all adult individuals ranged from 1 (maximum) to 0 (minimum). General pattern observed for both the centralities show that highest ranking adult females having higher centrality values, but the pattern is not consistent with intermediate and subordinate females, where the rank alone does not determine the influence of an adult individual on the troop. Few intermediate and subordinate individuals have high centrality values (Table 3.6).

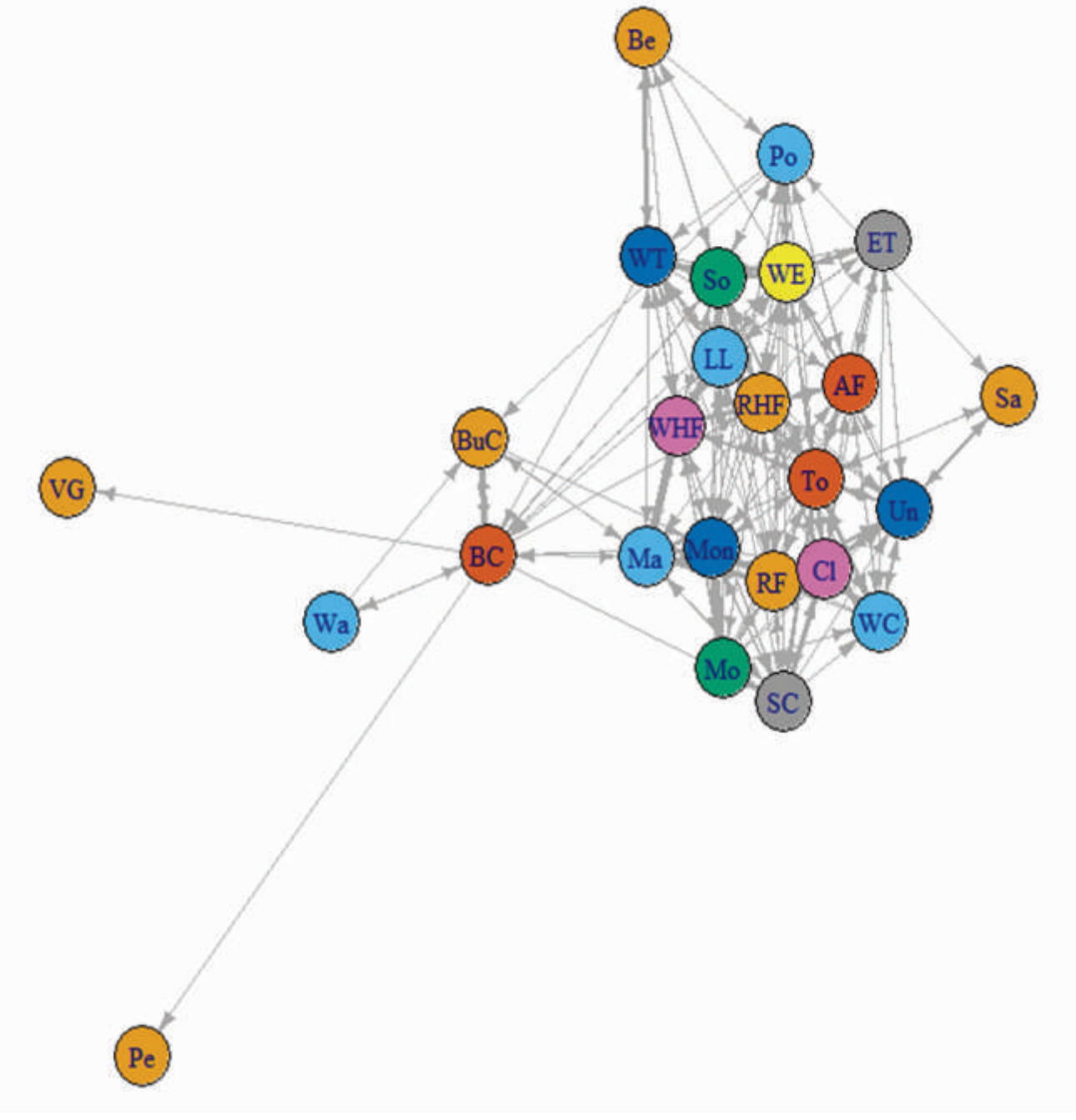


Figure 3.8: *Social Network of dyadic affiliative interactions of adult individuals in troop B.*

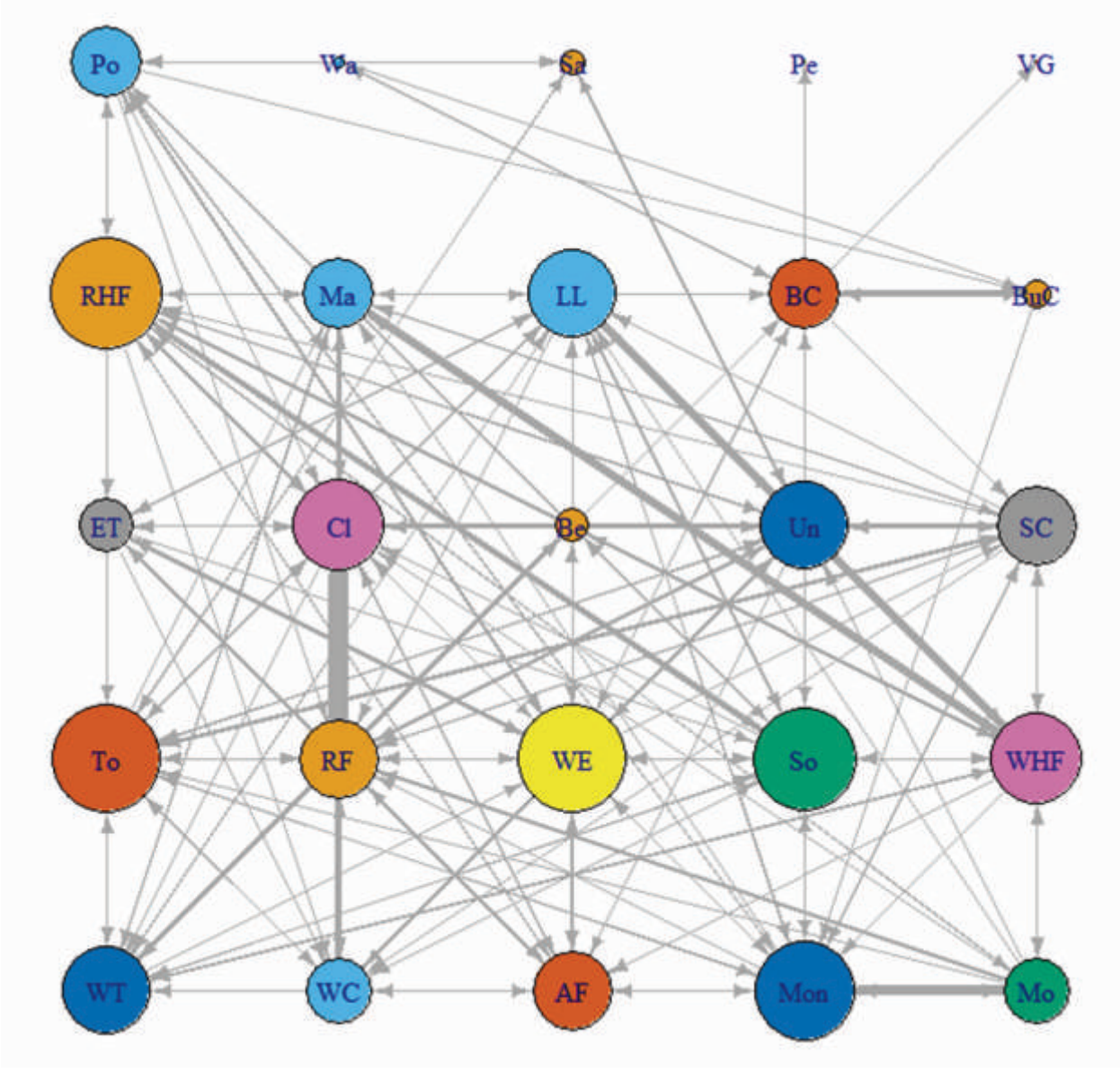


Fig 3.9: *Social Network of dyadic affiliative interactions of adult individuals in troop B where size of nodes represents total degree.*

Agonistic network

Figure 3.10 and 3.11 represents the social network for agonistic interactions. The nodes represent individual identities of adult male and adult females of the troop. Nodes are connected by edges, here edges represent the agonistic interactions, and the width of edges describe the intensity of these interactions. The arrows represent direction of agonistic interaction, which can be unidirectional or bidirectional. In Figure 3.11 the width of nodes represents out degree of agonistic interactions in adult macaques. The bigger the size of node, there are more agonistic interactions directed by that individual to other members of the troop. The values of out-degree ranges from 19 to 0. The out degrees for agonistic interactions are higher for dominant individuals and lower for intermediate and subordinate individuals.

Table 3.6: Eigenvector Centrality and PageRank Centrality values from affiliative network and out degrees from agonistic network of adult individuals in study troop B.

S. No.	Individuals	Eigenvector Centrality	PageRank Centrality	Out-Degrees
1	Red face	1	0.11	19
2	Ape face	0.293	0.046	15
3	Cleft	0.525	0.09	12
4	Sister Cleft	0.164	0.032	11
5	White eye	0.284	0.049	8
6	Unibrow	0.227	0.047	9
7	Tongue	0.191	0.036	14
8	Red HF	0.255	0.034	7
9	White HF	0.447	0.061	4
10	Long lash	0.321	0.04	2
11	Monroe	0.17	0.035	11
12	Molly	0.131	0.025	7
13	Polka	0.139	0.029	7
14	Ear tag	0.126	0.024	4
15	White collar	0.31	0.044	11
16	White tape	0.222	0.040	10
17	Black collar	0.063	0.035	4
18	Socket	0.231	0.036	6
19	Sally	0.059	0.016	0
20	Beardy	0.065	0.019	17
21	Matku	0.55	0.088	17
22	Blue collar	0.063	0.027	17
23	Thakur	0	0	4
24	Peripheral	0.002	0.007	7
25	Wart	0.006	0.009	4
26	Van Gogh	0.002	0.007	0
27	Kaptan	0	0	1

This implies that high ranked individuals show more agonistic behaviour towards other individuals of the troop.

Further, using the affiliative network will increase our understanding of social complexity in the macaque troop where highly central individuals are important to maintain group cohesion; these central individuals might aid in the successful integration of new individuals and influence others' individual welfare by decreasing social tension and individual levels of stress. Measures of network centrality and the identification of significant subgroups (or clusters) through SNA are important to consider when introducing new individuals, or determining who to transfer during management interventions for population control. While, agonistic networks will help identify key individuals who initiate and direct the aggressive behaviour in a troop. In the context of current study in an urban landscape, where rhesus macaques are in close contact

with humans, it becomes imperative to understand the flow of agonistic interactions whenever there are incidents of human-macaque conflicts. This can further aid in targeting individuals for behaviour interventions to reduce conflict at a later stage.

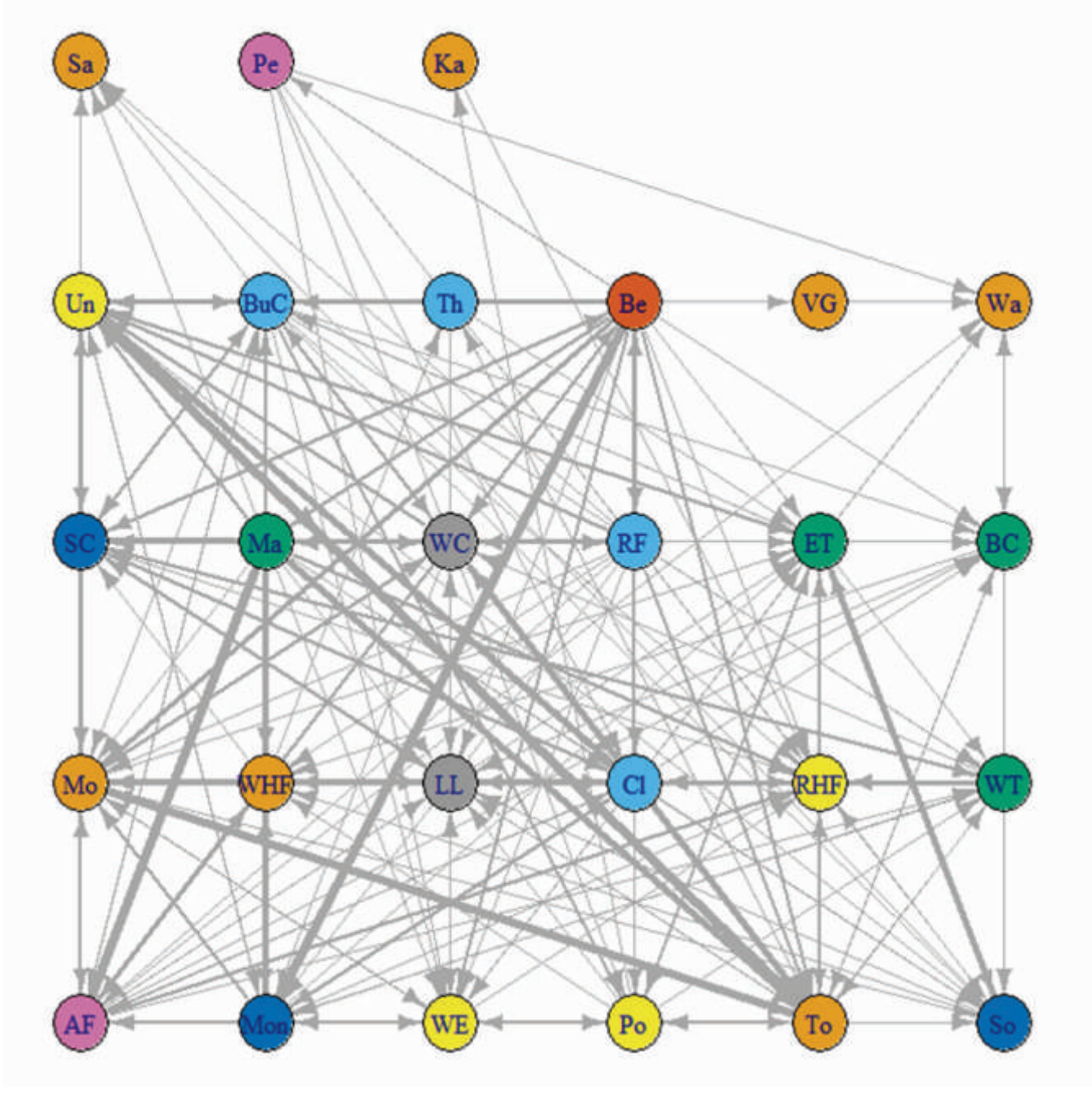


Fig 3.10: *Social Network of dyadic agonistic interactions of adult individuals in troop B.*

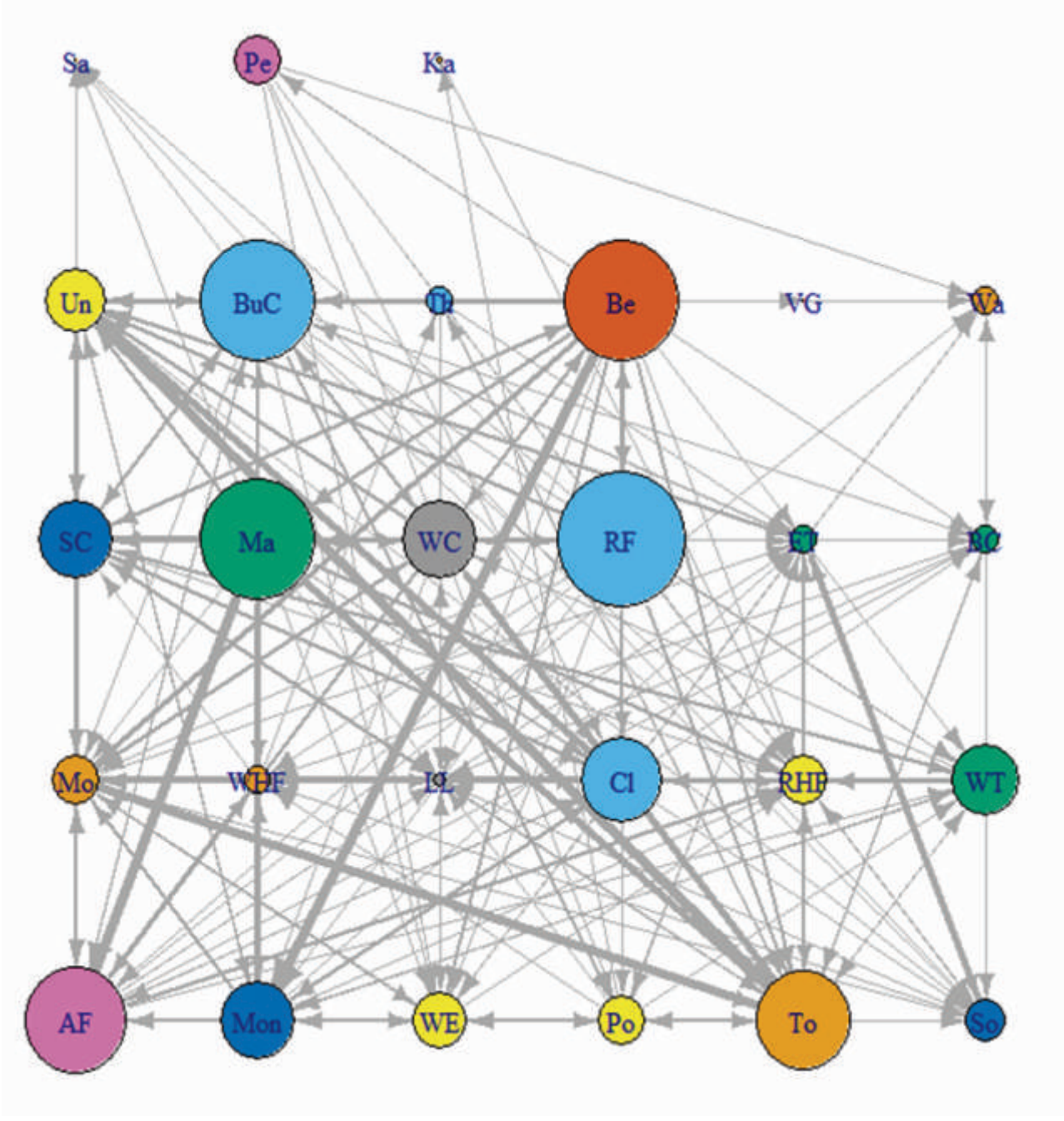


Fig 3.11: *Social Network of dyadic agonistic interactions of adult individuals in troop B where size of nodes represents out degree.*

SECTION II

Asian Elephant

(Elephas maximus)

Chapter 4

UNDERSTANDING HUMAN-ELEPHANT CONFLICT

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Historically elephants (*Elephas maximus indicus*) were distributed across India, starting from the southern Himalaya, excluding only the arid tracts in the country. The current distribution is however restricted to only the Himalayan foothills in the north, the hills of the Western Ghats in the south, forests of north-eastern states and the forests of east-central India (Baskaran et al., 2011; Sukumar, 2006). Current estimates indicate a countrywide population size of 27,000-28,000 in the wild (www.moef.nic.in). In the last few decades, human elephant conflict has received considerable attention by researchers, conservationists, and management alike. The human elephant negative interactions are nevertheless not new to India. For instance, the "Gajasastra", an elephant lore written in Sanskrit by Palakapya has records of crop depredation by elephants in the ancient Indian kingdom of Anga, as early as the sixth and fifth centuries BC (Sukumar, 2003; Wakankar and Mhaiskar, 2006). However, statistics indicate that the dynamics of such interactions between humans and elephants are becoming more hostile over the years, leading to increased loss of human life (> 400 deaths annually), crop-damage (~ 330 sq. km every year) and elephant deaths (100 annually in retaliation) (Rangarajan et al., 2010). Crop-raiding by elephants is considered to be one of the main issues that has created human hostility to elephants in Asia (Sukumar & Gadgil, 1988, Santiapillai & Widodo, 1993; Balasubramanian et al., 1995; Williams et al., 2001; De Silva & Srinivasan, 2019). The conflict has currently increased

to such an extent that more than half the expenditure incurred by the Project Elephant goes for the Human elephant conflict mitigation (ETF, 2010).

The causes of human–elephant conflicts are numerous, complex and not necessarily caused by high density or fast multiplication of elephants. Nonetheless, actively managing elephant population becomes imperative in certain circumstances where habitat conditions are saturated and unsuitable, wherein habitats may not support any further increase in the elephant numbers (Bertschinger et al., 2018). For example, there are certain pockets such as North and South Bengal region in West Bengal, Hassan and Kodagu districts in Karnataka and certain fringe areas in Chhattisgarh where the remnant habitat for elephants appears to be limited and even a marginal increase in elephant numbers have been posing huge difficulties to the management.

These human-elephant conflict prone areas in North Bengal and Karnataka have historically been fragmented with tea/coffee estates, and the extant elephant population is partially isolated within highly human dominated landscapes. There is substantive evidence that these local populations are causing unprecedented levels of human deaths and damage to property in these specific areas. For instance, between 2006-2016, a total of 2122 human casualties occurred in North Bengal region comprising of just three districts viz., Darjeeling, Jalpaiguri, and Coochbehar. Out of these, 476 persons died whereas the rest sustained substantial injuries due to elephant attacks (Naha et al., 2019). Similar observations have been done in certain regions of Karnataka. In Virajpet forest division alone (occupying the southern part of Kodagu circle, Karnataka), at total of 39 human casualties, and 5106 instances of crop raiding were witnessed by the forest department between 2013 – 2020, amounting to more than Three crore rupees as ex-gratia/ compensation payment (Table 4.1).

Similarly, in northern Chhattisgarh, elephants have recently re-colonized the landscape resulting in high-levels of conflict. It may be noted that despite supporting a small population of about 200 elephants (<1% of country's wild elephant population), northern Chhattisgarh suffers a disproportionately high level of conflict with over 60 (over 15%) human-elephant conflict related human fatalities reported in the country annually. These negative interactions

Table 4.1 : Crop damage, human fatality and animal death due to Elephant conflict and compensation paid to the Stakeholders in Virajpet Division, Kodagu, Karnataka from 2013 – 2020.

Year	Crop Damage		Human Death		Human Injury		Animal Damage	
	No.	Rs	No.	RS	No.	Rs	No.	Rs
2013-14	633	25,20,701.00	--	---	02	40,000,00	--	---
2014-15	780	24,67,667.00	--	---	01	15,000.00	--	---
2015-16	720	32,37,912.00	04	20,00,000.00	05	7,000.00	--	---
2016-17	419	14,67,180.00	04	20,00,000.00	02	1,28,073.00	01	7,000.00
2017-18	1151	54,23,531.00	03	13,00,000.00	03	37,460.00	02	20,000.00
2018-19	732	49,00,604.00	--	---	02	12,093.00	04	26,000.00
2019-20	671	63,31,986.00	--	---	04	1,63,016.00	02	17,000.00
Total	5106	2,63,49,581.00	11	53,00,000.00	19	43,62,642.00	9	70,000.00

have also caused elephant deaths at an alarming rate. The retaliatory killing of elephants between 2008–2011 in Karnataka stood at 101, most of which were concentrated over Hassan and Kodagu districts of the state (Gubbi et al., 2014). Thus, without timely resolution of this situation, people's support for elephant conservation will drastically deteriorate and be counterproductive to our goal of conservation of the species in India. With this context, motivation behind exploring immunocontraception as a tool for population management in elephants was to develop a long-term solution that is humane, efficient and reversible, only to be used in case-specific sites/population, as and when warranted, so as to keep conflict elephant population in these sites within the tolerable limits. This also reiterates the actions outlined by the Elephant Task Force, constituted by the Ministry of Environment, Forest and Climate Change, Government of India, which had compiled information critical to the survival of elephants in India in 2010.

4.1 Current status of human-elephant conflict in Karnataka

The state of Karnataka presently holds the largest wild Asian elephant population in India, and perhaps the single largest population of Asian elephants anywhere in the world, with about 5,300 to 6,200 individuals spanning over an area of 14,500 sq. km (MoEF, 2017; Krishnan et al., 2019). Being the “State Animal”, elephants are also largely used as a flagship species for wildlife conservation in Karnataka. The state, recognising its importance for elephant conservation, declared the formulation of Mysore Elephant Reserve in 2002, which is currently the largest elephant conservation landscape in the country. A matrix of dry and moist deciduous, semi-evergreen and evergreen forests along with multiple use agroforestry land constituting the buffer area (Gubbi et al., 2014). This, in addition to effective laws, their stringent implementation and protection offered by the government, general public tolerance and effective stakeholders' participation has ensured Karnataka to still hold the highest densities of elephants, thereby being a key for elephant conservation and the long-term survival of the species in the country (Gubbi et al., 2014).

Nonetheless, elephants are also the most conflict-prone wildlife species in the state. To elaborate, a study carried out over a span of 4 years, from April 2008 to March 2011, showed that out of the 42 forest administrative divisions in the state, 25 divisions (< 50%) had experienced HEC, resulting in 60,000 plus individual instances, amounting to ≈ 23 crores/2.9 million USD (Gubbi et al., 2014). A recent HWC study by Wildlife Institute of India (WII) however showed drastic increase in the HEC instances in the state, leading to the payment of ex-gratia amount of ≈ 32 crores (4.1 million USD) by the Forest Department of Karnataka (KFD) just between 2019-2021; almost twice the instances in half of the time compared to Gubbi and co-workers' study in 2011.

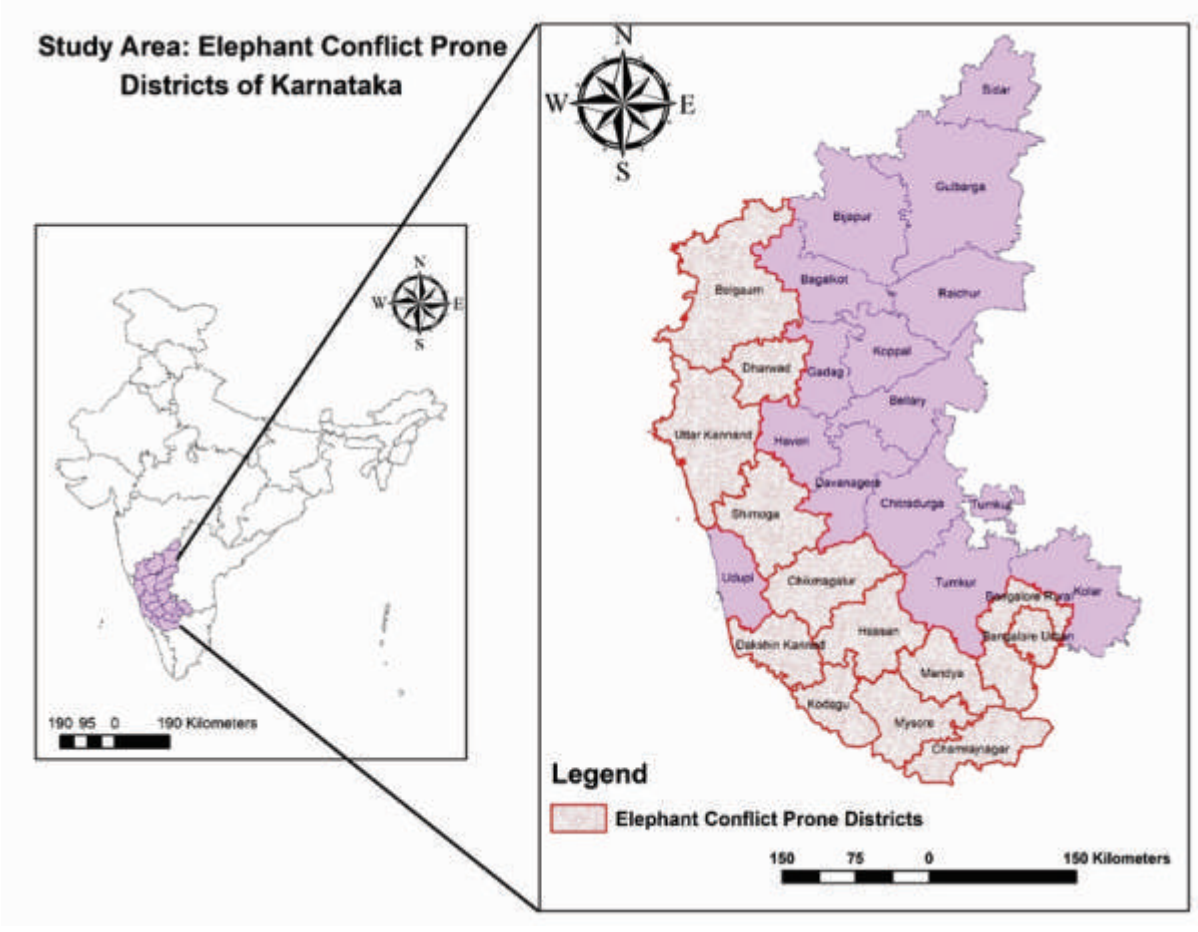


Figure 4.1 : *Elephant conflict prone regions in study area.*

The detailed figures of HEC instances during WII's study are placed in Table 4.1. Further, the study by Gubbi and co-workers depicted highest HEC instances in southern Western Ghats, and almost non-existence of HEC in the central Western Ghats landscape in Karnataka. Though southern Western Ghats still faces highest HEC instances in the state, both central and northern western Ghats have also started exhibiting increasing trend in HEC instances as depicted in figure 4.2 & 4.3 and table 4.2 (Qamar Qureshi, unpublished data). The HEC instances also seem to have spread to novel areas, with increased occurrences now being reported from districts such as Belgaum, Dharwad, and Haveri distracts in the North and Kolar, Ramanagara, Mandya, and Dakshina Kannada, in the south (Figure 4.1).

The data were collected in collaboration with the Karnataka State Forest. We compiled data from the Karnataka state forest department compensation records between 2019–2021. Based on this secondary data, we documented the nature of such incidents and the extent of Human-wildlife conflict. All these conflict locations were later mapped in Arc GIS 10.2.5. Hotspots of human-elephant conflicts were identified concerning conflict locations across Karnataka. A considerable amount of crop and property damage occurs with State Government spending substantial funds in controlling such depredation events and paying ex gratia / compensation to affected people. The hotspot maps of conflict risk will help in developing

appropriate mitigation strategies such as setting up early warning systems, restoration of wildlife corridors, using deterrents and barriers for the vulnerable (Naha et al. 2018).

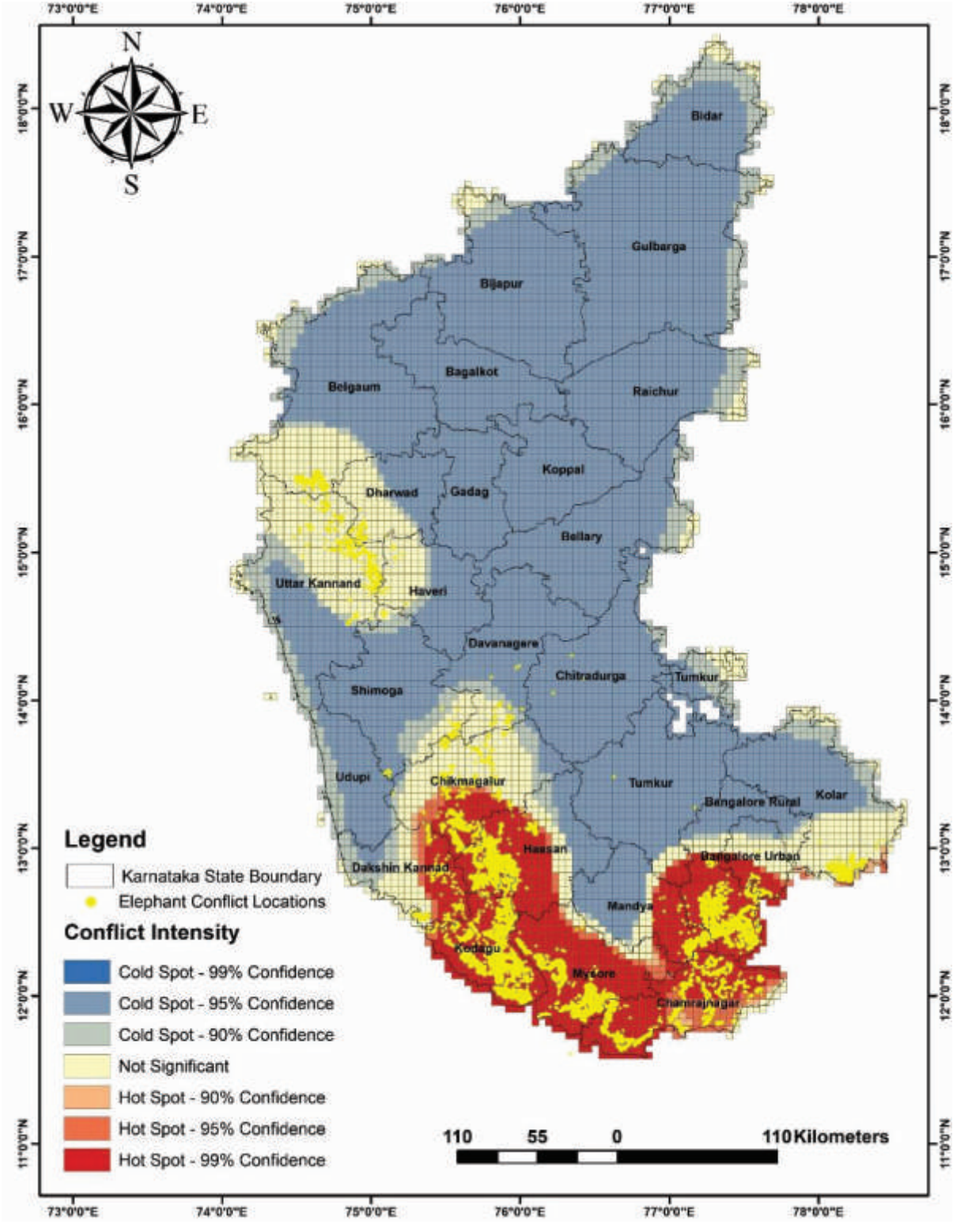


Figure 4.2 : *Human-elephant conflict Hotspots in Karnataka based on HEC instances documented by Karnataka Forest Department between 2019 -2021 (Data collated from e-parihara through MoEFCC-HWC project, 2021).*

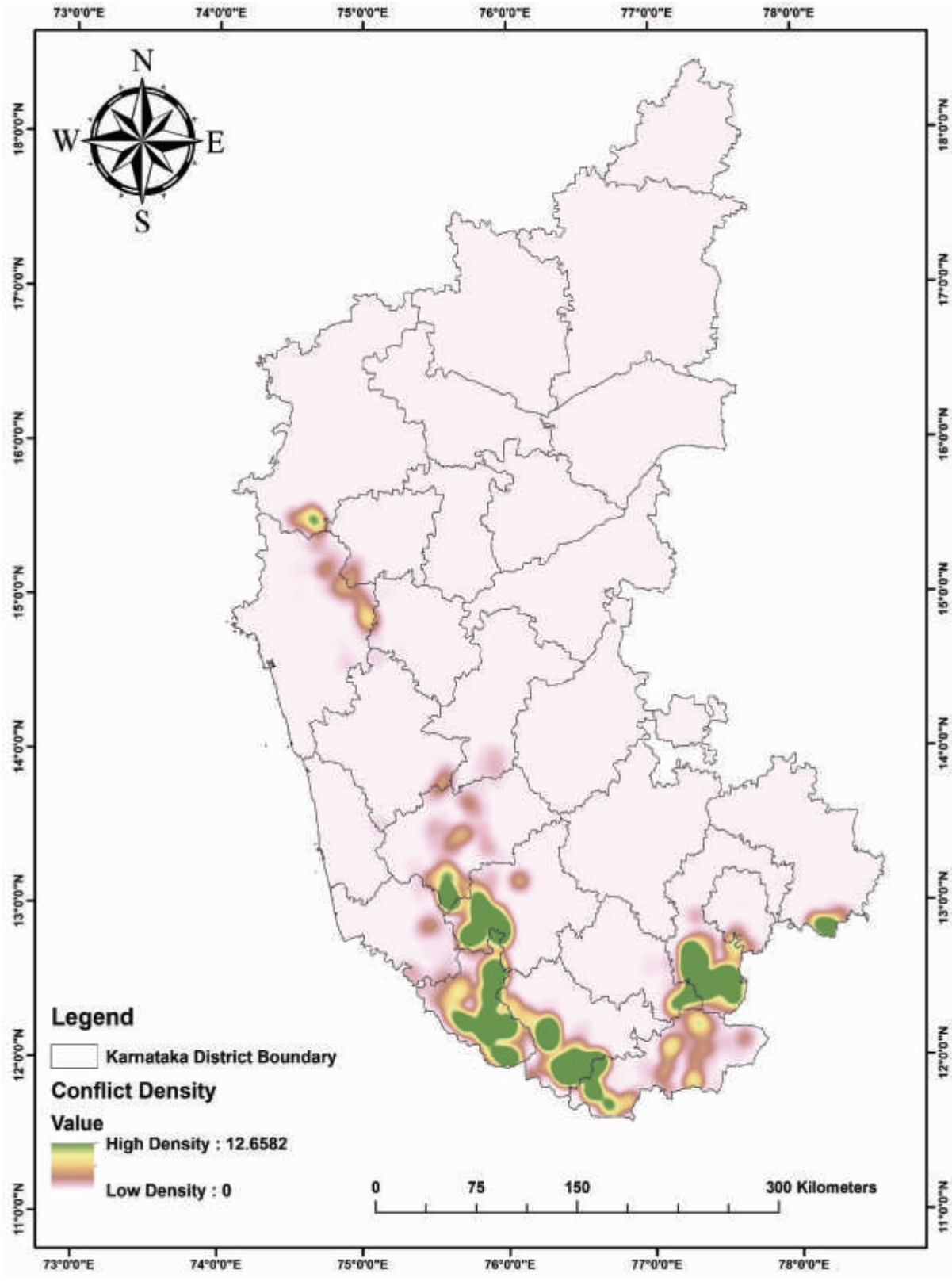


Figure 4.3 : *Human-elephant conflict Density in Karnataka based on HEC instances documented by Karnataka Forest Department between 2019 -2021 (Data collated from e-parihara through MoEFCC-HWC project, 2021).*

Table 4.2 : Forest Administrative Circle wise data on HEC and Ex-gratia in Karnataka from 2019-2021 (Data collated from e-parihara platform through MoEFCC-HWC project, 2021).

	Crop Damage		Property Damage		Human Death/Injury		Livestock Death/ Injury	
Forest Circle	**Total No of Cases**	**Compensation paid in Rupees**	**Total No of Cases**	**Compensation paid in Rupees**	**Total No of Cases**	**Compensation paid in Rupees**	**Total No of Cases**	**Compensation paid in Rupees**
Bangalore	7700	118504289.2	799	5320775	13	4180000	17	170000
Belgaum	545	3444578.1	1	10000	NA	NA	NA	NA
Chamarajanagar	3315	18925685.06	330	2479386	7	1619542	4	35000
Chickmangalur	2163	18172022.9	36	331900	2	60000	6	60000
Dharwad	173	907456.04	11	71800	NA	NA	NA	NA
Hassan	4081	27332661.5	132	1087000	4	840000	5	45000
Kanara	832	7364316.14	5	45000	2	60000	NA	NA
Kodagu	9749	59481122	455	3809047	22	6891877	7	90000
Mangalore	283	3639333.2	33	175140	NA	NA	NA	NA
Mysore	6069	23009924.57	371	1804468	8	123796	10	88000
Shimoga	352	2725830.76	6	57000	1	30000	NA	NA
Bellary	4	38620	NA	NA	NA	NA	NA	NA
Total	**35266**	**28,35,45,839.5**	**2179**	**1,51,91,516**	**59**	**1,38,05,215**	**32**	**3,18,000**

A landscape level approach, including increasing habitat quality and connectivity, protection of existing elephant corridors and creation of new ones wherever deemed necessary is indeed the pertinent long-term solution to mitigate HEC. However, in certain high conflict areas in Karnataka that have historically been fragmented with agroforestry practices, regaining natural land and conversion to refuges/corridors is unlikely to happen instantaneously. Additionally, a significant population of elephants currently live outside protected areas in Karnataka, again owing to prolonged anthropogenic causes such as land use modifications. A sizable human population also resides either inside or at the fringes of such elephant habitats, leading to intense negative interactions with elephants (Krishnan et al., 2019). Thus, steps need to be taken simultaneously to address the issue until habitat modifications are achieved, to contain HEC within tolerable limits and in turn safeguard community support towards elephant conservation.

Situated on the top, the eastern and western slopes of the Ghats, Kodagu district occupies about 4,102 sq. km (1,580 miles) area in the Western Ghats on the south-western border of Karnataka between 11˚ 56' and 12˚ 52'N latitudes and 75˚ 22' and 76˚ 11'E longitudes. It is bounded by Hassan district on the North, Mysore district on the East, on the West by Dakshin Kannada district, all in Karnataka and in the South by Kannur district in Kerala. While the northern and the western parts have evergreen forests, the moist deciduous and dry deciduous forests are found in the central and southern parts of the district. Isolated evergreen and Shola forests are also found nestled between the folds of the mountain slopes which are covered by grasslands. The district has approximately 3,263 sq.km of forest cover out of its total geographical expanse, of which 795.90 sq.km has very dense forest, 1,888.21 sq.km has moderate dense forest while approximately 579.27 sq.km constitute open forests type (Forest Survey of India, 2019). Kodagu being located in Central Western Ghats also houses a great number of species that are endemic to the Ghats. The district has one tiger reserve and three

Table 4.3 : Population of elephants in different forest divisions of Karnataka (Varma and Sukumar 2012)

S.No	Forest Division	Area (Sq.km)	Number	95% CI (LCL– UCL)
1	Madikeri territorial	1052	273	176 – 369
2	Madikeri wildlife	344	192	118 – 266
3	Nagarahole	643	1320	950 – 1690
4	Virajpet	116	65	46 – 84

wildlife sanctuaries; the Nagarhole tiger reserve, Talacauvery, Pushpagiri and Brahmagiri Wildlife Sanctuaries respectively. These in addition to surrounding protected areas extend up to the Eastern Ghats in a continuous belt that holds the single largest contiguous population of Asian elephants, with an estimated 9,000 individuals (Sukumar 1989; Vidya et al. 2005). The region has 1–3 elephants per sq.km, one of the highest elephant densities in Asia (Kemf and Santiapillai 2000). Though the robust account on elephant numbers in the district is not available, estimates based on the direct (sample block count) method as recommended by Project Elephant Directorate, table 4.3 reports the elephant estimates.

Kodagu is among the biggest producer of coffee in India and in fact, around 40% of India and 2% of the world's coffee is produced here (Coffee Board of India, 2008; Bal et al., 2008). According to the Coffee Board of India in the period of 2018-19 around 16900 metric tonnes of Arabica and 93,830 metric tonnes of Robusta were produced amounting to a total of 110,730 tonnes for the district of Kodagu (Coffee Board of India, 2020). Paddy cultivation was one of the main sources of income in Kodagu until coffee boomed as the major commercial crop and was developed in a larger area. In addition to State owned forests, Kodagu landscape comprised of evergreen and moist deciduous forest patches, owned by community or private property, interspersed with agricultural areas (Ramakrishan 2000). Nonetheless, due to the commercially driven coffee market dynamics, coffee cultivation was intensified and from 1977 to 2007, Kodagu lost 30% of its natural forest cover whilst the area under coffee increased by 100% (Elouard and Guilmoto 2000; Bal et al., 2011b). Even though the forest areas controlled by the Karnataka Forest Department (KFD) had remained largely intact, the plantations had predominantly expanded into privately owned forests leading to massive biodiversity depletion and landscape alteration. While this led to massive alteration in forest diversity and degradation of floral and faunal species distribution, the canopy cover remained hardly altered and about 60% of Kodagu is still covered with shade grown coffee plantations and other crops like cardamom, thus resembling natural forest for an elephant (Narayana, 2014).

Additionally, certain large coffee holdings are hardly situated 5-10 km from major protected areas in the district and have abundant water sources for irrigation, edible tree species and have green cover throughout the year, even in dry season. Water bodies found in such estates are perennial as compared to seasonal water bodies in adjacent protected areas and neighbouring agricultural landscapes also provides dense, highly palatable and accessible resources such as paddy, grass, water, fruit trees, coffee, etc, all of which act as an attractant for local elephant population. These estates are very often connected by undisturbed privately owned forests, community owned forests or sacred groves (Narayana, 2014). Additionally, such

estates have low human population density which implies that large portion of estate can remain undisturbed for days or even weeks. The combined effects of all these factors have not only made usage of these estates as temporary migratory routes for elephant herds (Nath and Sukumar, 1998), it is also now being increasingly used as permanent refuge sites by certain elephants outside protected areas.

The combined effects of high elephant density and major landscape changes are thus perceived to be the key cause of human–elephant conflict in the landscape (Bal et al., 2008). Further, according to one of the surveys carried out in the landscape, causes like presence of insufficient natural resources to sustain the large elephant population in protected areas of the landscape, and exhibition of novel behavioural patterns among the resident elephants have also been reported to have caused increased conflict in the area (Bal et al., 2011). While most of the studies on elephants in the landscape have aimed toward quantifying the human elephant conflict, there have been minimal studies to understand the drivers leading to the issue. There is still an acute paucity of information on the distribution, demographic attributes, movement or behavioural patterns of the elephant population in Kodagu and evidence of how this mixed-matrix of landscape is being used as corridor during migration or as a refuge by the local elephant population is yet to ascertained (Narayana, 2014).

The project team, has thus initiated a long-term study in Kodagu landscape to ascertain above attributes and fill in the knowledge gaps in a scientific manner. As it is important to ascertain the population and demography characteristics of elephants in the region before deciding upon management interventions, extensive field work in Kodagu was initiated with establishment of a base camp in Virajpet Division in November, 2019. Subsequently, a stakeholder meeting and an initiation workshop was also organized to sensitize the local forest department staff on project objectives and methodology in December, 2019.

4.2 Monitoring damage caused by elephants in Virajpet Taluk, Kodagu

In order to implement population control strategies, it is imperative that information on both species specific and target site specific socio-economic factors, including the frequency and severity of conflict and ensuing economic losses, human health (physical and mental) problems and environmental effects are known. Thus, available elephant conflict data from 2015 – 2020 with the forest department was collected and compiled to ascertain the actual status of HEC in Kodagu study area. Preliminary analysis indicates a wide variety of crops being damaged by elephants in the area, including areca nut, beans, banana, cardamom, coconut, coffee, cotton, ginger, maize, mango, orange, paddy, pepper, ragi, sapota, silver oak and toddy palm with the total compensation being paid between 2015-2020 exceeding 6 crore rupees (Figure 4.5 & 4.6, and Table 4.4 & 4.5). During the period, a total of twenty-four human casualties occurred due to elephants, out of which thirteen resulted in human death in the study area.

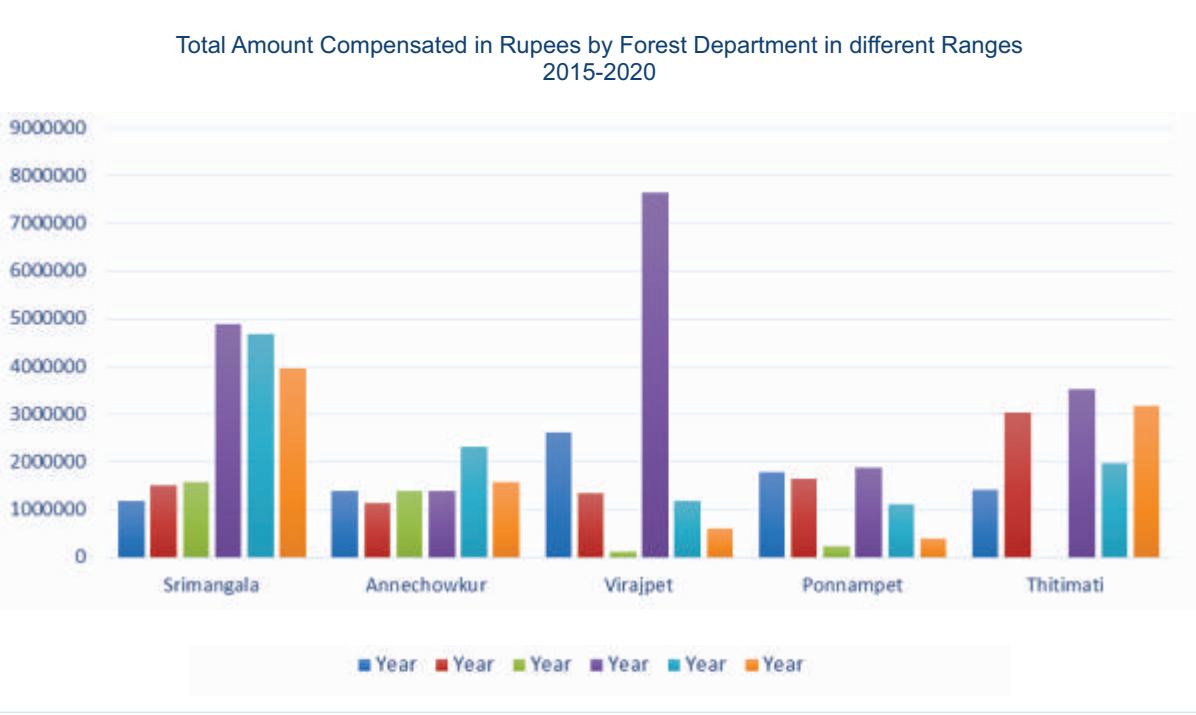


Figure 4.4 : *Range wise compensation paid to the Stakeholders in study between 2015– 2020.*

Table 4.4 : Crop wise compensation paid (INR) to the Stakeholders in various ranges of study area in Kodagu, Karnataka from 2015– 2020.

Range	Areca nut	Banana	Cardamom	Coconut	Coffee	Ginger	Orange	Paddy	Pepper	Sapota	Cotton	Mango	Silver Oak	Toddy palm
Virajpet	1468870	2487027	36560	924470	3866358	32190	320	2427530	201010	0	0	0	0	0
Thitimathi	1641070	1976358	47400	978302	5128790	129405	83620	1826456	699890	0	0	0	0	0
Ponnampet	658500	1657872	0	443287	1439200	40910	0	1095155	55350	0	0	0	0	0
Annechowkuru	2599946	398376	0	830160	2923749	85212	160422	1053252	272140	26800	1000	2400	66000	67400
Srimangala	5808900	3237480	50600	830000	6464243	79396	21240	1261504	80850	400	0	0	0	0
Total	12177286	9757113	134560	4006219	19822340	367113	265602	7663897	1309240	27200	1000	2400	66000	67400

Table 4.5 : Property damage wise compensation paid (INR) to the Stakeholders in various ranges of study area in Kodagu, Karnataka from 2015– 2020.

Type of Property	Srimangala	Thitimathi	Virajpet	Ponnampet
Irrigation Pipe	1247000	501600	314240	45150
Gate	30000	106150	29312	0
Solar Fence	10000	5840	4300	0
Fencing Cement Pillar	10000	0	6100	0
Irrigation Motor	13000	0	0	0
House/Compound Wall	25000	37930	35500	3200
Pond	0	10400	22780	0
Vehicle	0	45000	9000	5000
Others(Cements,fertilizers,sheets,sprinklers,drums,tank,shed,tiles)	34950	109075	66000	12100
Total	1369950	815995	487232	65450

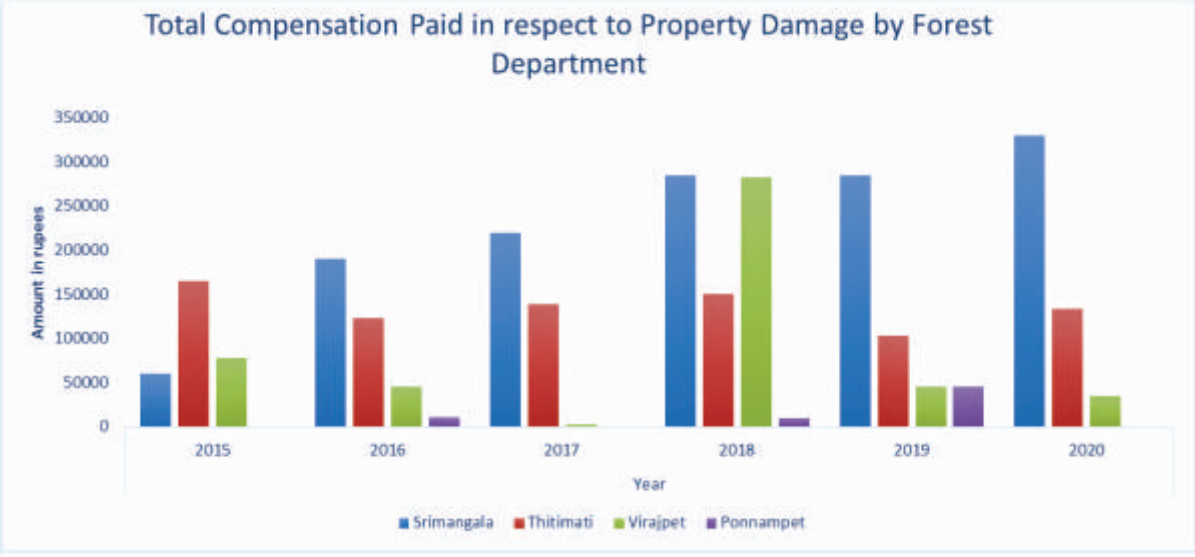
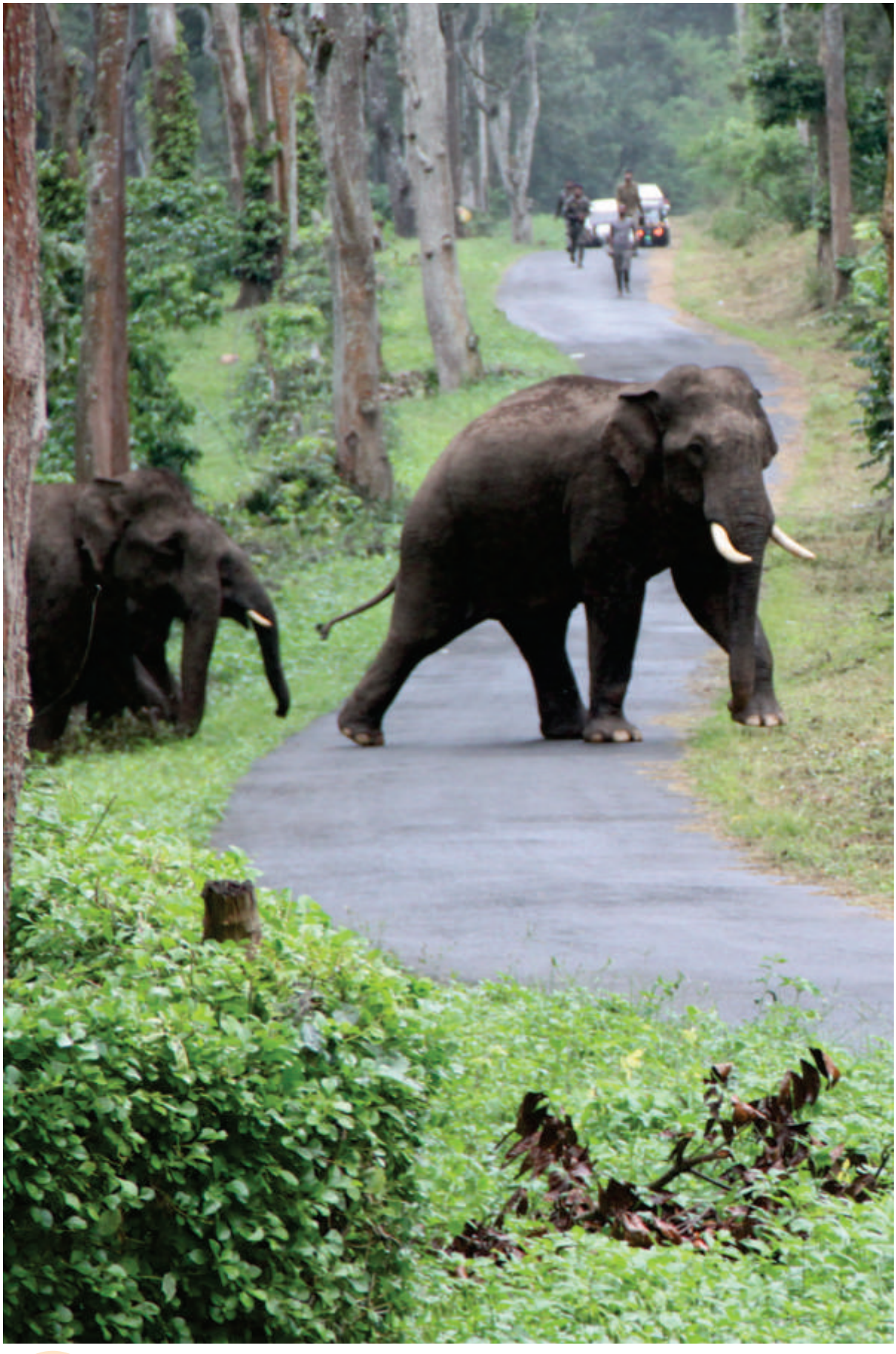


Figure 4.5 : *Total Compensation Paid in respect to Property Damage by Forest Department in various ranges of study area in Kodagu, Karnataka from 2015– 2020.*



Chapter 5

POPULATION ESTIMATION OF ASIAN ELEPHANT IN KODAGU, KARNATAKA

Vishnupriya Kolipakam, Mayur Vikas Markad, Souritra Sharma, Thammaiah Chekkera Kuttappa, Sanath Krishna Muliya, Farha Usmani, Bhawana Pant, Bhim Singh, Lallianpuii Kawlni, Rochitha Shree, Amritesh Ranjan Dubey, YV Jhala, Qamar Qureshi

5.1 Abundance estimation of Elephants

Estimating the abundance of Asian elephants is a complex task that requires the application of various methods and techniques. One commonly employed approach is direct observation, where trained observers physically count the number of elephants within a given area. These surveys involve walking or driving through the habitat and recording the elephants encountered. However, this method may face challenges when dealing with large or dense populations. Genetic analysis provides a non-invasive means of estimating population abundance and genetic diversity. By collecting dung samples and extracting DNA, researchers can identify individual elephants and gain insights into relatedness among individuals. Line transect surveys involve walking along predetermined transect lines and recording signs of elephants, such as footprints, dung, or feeding evidence. Statistical models are then used to estimate population abundance based on the collected data. It is important to note that due to the challenges posed by the large home ranges and elusive behaviour of Asian elephants, a combination of different methods is often used to obtain more accurate estimates. Collaboration with local communities and conservation organizations, as well as the incorporation of local knowledge, also plays a crucial role in gathering information about elephant populations.

5.1.1 Line transect method for estimating elephant dung density in Kodagu district of Karnataka

Monitoring animal population size, structure, and trend is an essential part of wildlife biology. However, such monitoring poses serious challenges when the animals of interest are difficult to detect because they are rare or elusive, live in concealing environments, or are sensitive to disturbance (Hedges et.al, 2012). The dung method is commonly used for estimating the density of elephants. Barnes, 1995 estimated the population density of elephants in Gabon. So, we tried to use the same line transect model for our study area to assess elephant density.

The transects were walked on a compass bearing counting all dung piles seen by measuring the distance from the survey line to the dung pile. The dung count-based survey was done from 27th December 2019 to 14th February 2020, to determine elephant dung-pile density (Buckland et al., 2001; Hedges and Lawson, 2006). For estimating elephant dung density, the dung bolus piles that were observed were recorded. During the line transect-based survey, each transect has been walked once and the data was compiled and converted to a text (tab-delimited) file to estimate dung density using a program in the Distance 7.3, which is based on the modelling of the probability of detection (Thomas et al, 2010).

The density of dung piles is calculated by:

$$D = \frac{N \times \bar{X}}{\sum l_i \times 2(W_{max} \times \beta)} \times \text{Area of region}$$

Where N is the number of groups, $\bar{X}$ is the mean group size, $\sum li$ is the summation of the transect length, Wmax is the maximum width up to which animals are recorded, β is the detection probability, and (Wmax x β) is the effective width strip. 1267 elephant dung piles along 40 transect with a total length of 182.30 km.

A total of 40 transects were walked in 5 different ranges of the Kodagu area covering Nagarhole Range (6), Annechowkur Range (10), Kallahalla Range (10), Srimangala (9), Mahakutta (5). Estimating elephant density from the dung-pile density requires data on rates of elephant defecation and dung-pile decay. We analyzed transect data using the program Distance (Thomas et al., 2010), to calculate dung density in our study area of Kodagu district of Karnataka.

Result

During the line transect-based survey, each transect has been replicated to increase sample size which is often essential, the transect is entered in the software Distance, with effort recorded as line length times the number of times the line. We can deduce elephant dung density by using distance software and by applying a mean defecation rate and decay rate, we can estimate elephant population density and population size of elephants in the Kodagu district of Karnataka.

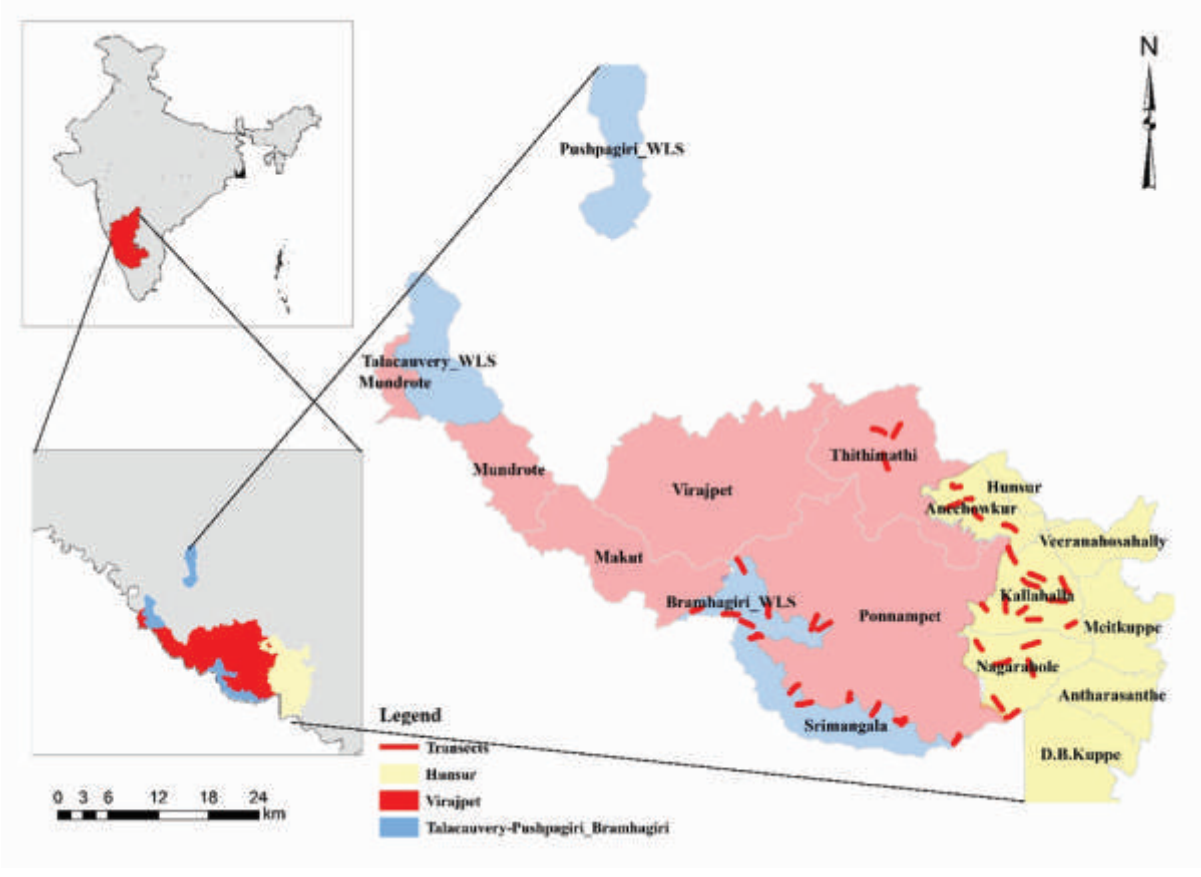


Figure 5.1 : *Transects walked in different ranges of Kodagu District*

We applied several models to deduce the density of dung piles and the best fit model with the lowest AICc value was the Half normal Cosine model by making necessary cut-points of the interval to fix the curve and after truncation to make the model the best fit. We estimated the dung density as 5718.340 (95% CI = [4281.281, 7637.765]) using the dung count method in Distance software and the best model (Half normal cosine model) has been selected on the basis of minimum AICc value (1384.826), giving an effective strip width of 2.47 meters, and p (detection probability) of 0.206. The percentage of CV (Coefficient of Variation) was reported to be 14.5% with group size (DS) of 1404.2 [LCL-1053.0, UCL-1872.6]

The elephant density can be calculated by (Dung density* Decay Rate)/ Defecation Rate. The decay rate and defecation rate were taken from the published literature to estimate elephant density. The Elephant density was calculated as 344/ 100 sq. km, where the decay rate was taken to be 0.0097 and the defecation rate as 16.33 (Watve 1992; Varman et. al. 1995).

5.1.2 Standardising methods for abundance estimation of elephants using Genetic mark recapture methods

The main aim of this exercise was to develop a correction factor for existing abundance estimation of elephants using DNA based mark-recapture. To achieve this objective, we aim to a) to establish a panel of microsatellite markers for identification of individual elephants, b) to

cross validate DNA extracted from 'Dung' and 'Blood' samples in identification of individual elephants and c) test the population estimate using Genetic capture-mark-recapture (CMR) method in a known population and further d) estimate the abundance of elephants from select field sites of Karnataka. For the standardization of molecular markers, work was carried out in Dubare and Mattigodu semi captive elephant camps of Karnataka as the population size is known and error rates, if any, while using the genetic mark-recapture method can be quantified.

A total of 35 blood and dung samples have been collected from Dubbare, Mathigodu and Doddaharve camps were collected. For the isolation of DNA, several manual and kit-based protocols were tested. Finally, DNA was isolated using silica column-based kit method (Qiagen). A panel of Twenty-two already published microsatellite markers viz., EMX-1, LA3, LA4, LA5, LA6, EMU01, EMU02, EMU03, EMU04, EMU06, EMU07, EMU08, EMU09, EMU10, EMU11, EMU12, EMU13, EMU14, EMU15, EMU17, EMU18 and EMU19 as reported by Eggert et al. (2000) and Kongrit et al. (2008) were initially tested to understand the success of individual identification. This panel has been narrowed down to ten loci (LA04, EMU13, EMU07, EMU08, EMU14, EMU18, EMU02, EMU01, EMU11, and EMU03) for individual identification from blood and dung samples, and we were able to assign the samples to different elephants.

Table 5.1 : Details of microsatellite marker panel for individual identification of Asian elephants. Highlighted set of markers are been used for individual identification

S.No.	Primer Name	Repeat Motif	Reference
1	EMX1	di	Eggert et al., 2000
2	LA3	di	Eggert et al., 2000
3	LA4	di	Eggert et al., 2000
4	LA5	di	Eggert et al., 2000
5	LA6	di	Eggert et al., 2000
6	EMU01	di	Kongrit et al., 2008
7	EMU02	di	Kongrit et al., 2008
8	EMU03	di	Kongrit et al., 2008
9	EMU04	di	Kongrit et al., 2008
10	EMU06	di	Kongrit et al., 2008
11	EMU07	di	Kongrit et al., 2008
12	EMU08	di	Kongrit et al., 2008
13	EMU09	di	Kongrit et al., 2008
14	EMU10	di	Kongrit et al., 2008
15	EMU11	di	Kongrit et al., 2008
16	EMU12	di	Kongrit et al., 2008
17	EMU13	di	Kongrit et al., 2008
18	EMU14	di	Kongrit et al., 2008
19	EMU15	di	Kongrit et al., 2008
20	EMU17	di	Kongrit et al., 2008
21	EMU18	di	Kongrit et al., 2008
22	EMU19	di	Kongrit et al., 2008

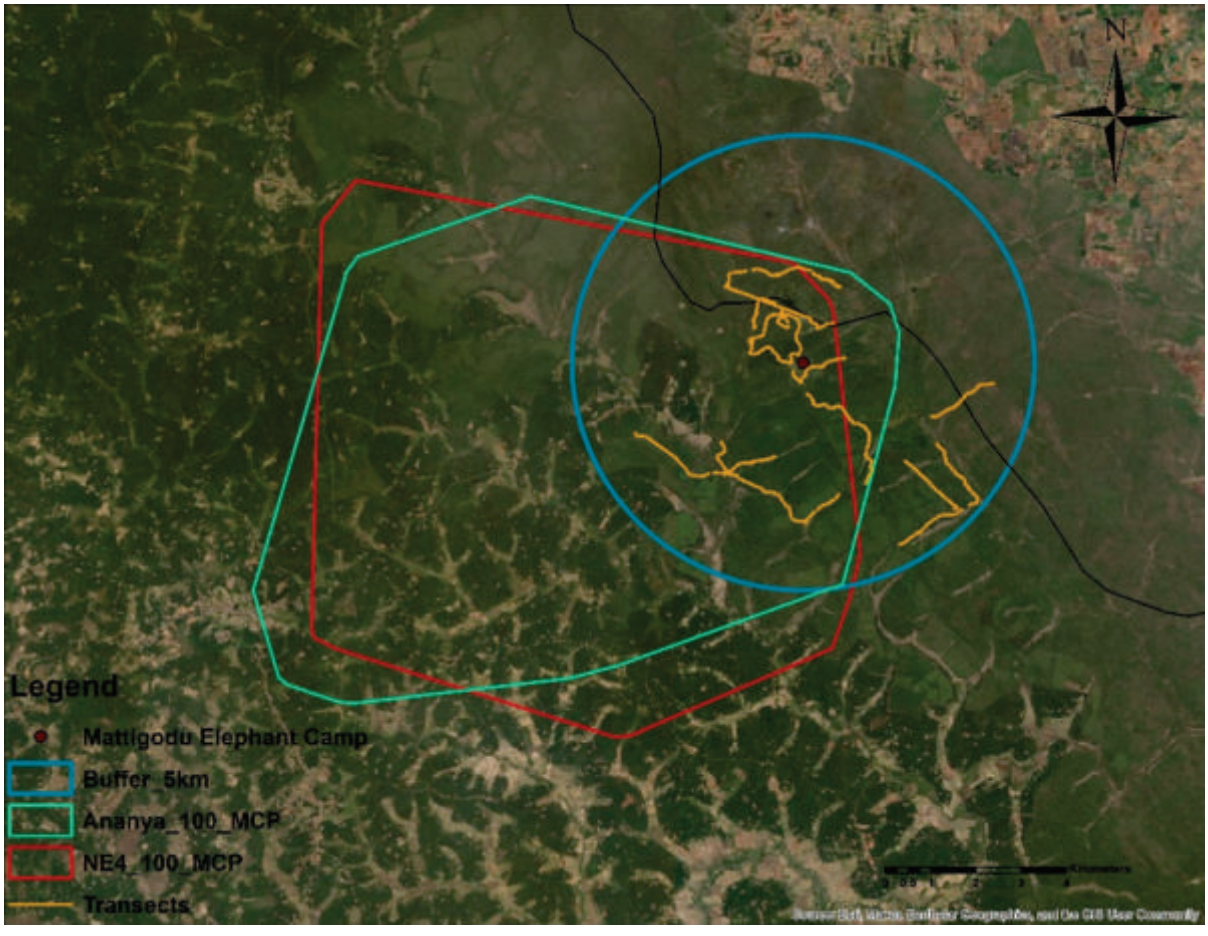


Figure 5.2 : *Map showing Mathigodu camp, line transect, a buffer of 5km radius, home ranges of Ananya (green) and NE4 (red).*

Dung samples for the blind test, to understand the efficacy of the standardized protocol are collected in Polygon search method (Efford, 2015), Nineteen line transects of approximately 2 km were walked within the 5 km radius of Mathigodu Elephant Camp a total of 138 dung samples were collected for CMR study. The transects were walked on a compass bearing counting all dung piles seen by measuring the distance from the survey line to the dung pile. The dung count-based survey was done from 21.06.2021 to 12.07.2021, to determine elephant dung-pile density (Buckland et al., 2001; Hedges and Lawson, 2006).

Result

The PCR amplification of DNA isolated from blood sample and dung sample showed similar results. Which indicates that purity (260/280 nm ratio was 1.7 to 1.8 nm and 1.3 to 1.98 nm for blood and dung respectively) didn't interfere with amplification of DNA. All the ten microsatellite loci used for DNA isolated from the blood and dung were found to be polymorphic. The total combined Probability of identity across all the loci was found to be 4 x 10-8 and 1.5 x 10-6 for blood and dung samples respectively, indicating a very small probability of wrongly identifying two different animals as the same animals.

Table 5.2 : Observed and expected heterozygosities, PIC, PID and F(Null) values of ten microsatellite loci for DNA isolated from blood

Locus	Ho	He	PIC	PID	F (Null)
LA04	0.676	0.673	0.589	0.188	-0.014
EMU13	0.970	0.675	0.601	0.176	-0.202
EMU07	0.765	0.650	0.593	0.176	-0.106
EMU08	0.912	0.651	0.568	0.202	-0.207
EMU14	0.588	0.596	0.550	0.208	0.012
EMU18	0.912	0.714	0.648	0.144	-0.137
EMU02	0.706	0.641	0.559	0.208	-0.083
EMU01	0.345	0.763	0.707	0.106	0.367
EMU11	0.559	0.532	0.475	0.276	-0.053
EMU03	0.500	0.651	0.574	0.196	0.121
Mean±SE	0.693±0.06	0.654±0.019	0.586 ± 0.019	-	-
		Combined PID		4x10-8	

Note: Ho= Observed Heterozygosity, He= Expected Heterozygosity, PIC= Polymorphic information Content, PID= Probability of identity and F(Null)= Frequency of null allele.

Table 5.3 : Observed and expected heterozygosities, PIC, PID and F(Null) values of ten microsatellite loci for DNA isolated from Dung

Locus	Ho	He	PIC	PID	F (Null)
LA04	0.471	0.585	0.504	0.250	0.106
EMU13	0.600	0.571	0.495	0.258	-0.070
EMU07	0.600	0.701	0.631	0.152	0.064
EMU08	0.935	0.588	0.489	0.267	-0.258
EMU14	0.929	0.638	0.550	0.216	-0.216
EMU18	0.853	0.627	0.546	0.218	-0.184
EMU02	0.643	0.527	0.416	0.334	-0.116
EMU01	0.227	0.449	0.405	0.349	0.326
EMU11	0.600	0.458	0.384	0.369	-0.166
EMU03	0.389	0.579	0.473	0.281	0.199
Mean±SE	0.624±0.073	0.572±0.024	0.489±0.023	-	-
		Combined PID		1.5x10-6	

Note: Ho= Observed Heterozygosity, He= Expected Heterozygosity, PIC= Polymorphic information Content, PID= Probability of identity and F(Null)= Frequency of null allele.

Data analysis

Out of 138 dung samples collected for density estimation by the genetic-CMR framework only 115 samples that could be genotyped successfully corresponded to 21 unique individuals with a detection probability of 3 to 4 dung samples/ km. Using the SECR analysis we obtained the density estimate of 13.64 ± 3.68 elephants per 100 Sq. km. with Upper Confidence Limit (UCL) and Lower Confidence Limit (LCL) of 22.7, and 8.2, respectively. The detection probability at the home range centre was lambda = 0.48 ± 0.10 and the scale parameter was sigma= 21.00 ± 2.77.

As the total area accounted for the elephants to estimate the abundance is 165.63 km. The abundance is calculated from density per sq. km obtained from SECR. (Density x Area) which gives us results of 23 animals [LCL-13, UCL-38]. So, the known population is 34 elephants (Mattigodu camp-12 semi captive elephants and the combined group size of NE4 and Ananya is 22) which lies within our confidence limit of 13 to 38 elephants as calculated from the SECR density estimates.

We applied several models to deduce the line transect based density of dung piles and the best fit model with the lowest AICc value was the Half normal Cosine model by making necessary cut-points of the interval to fix the curve and after truncation to make the model the best fit. The elephant dung density was found to be 7264.021 [LCL- 4710.42, UCL- 11201.98] with an AICc value of 383.12 with group size (DS) of 4045.7 [2643.9, 6190.7], giving an effective strip width of 0.683 meters, and p (detection probability) of 0.341. The percentage of CV (Coefficient of Variation) was reported to be 21.2%. The elephant density can be calculated by (Dung density* Decay Rate)/ Defecation Rate. The decay rate and defecation rate were taken from the published literature to estimate elephant density. The Elephant density was calculated as 431/100 sq. km, where the decay rate was taken to be 0.0097 and the defaecation rate as 16.33 (Watve 1992; Varman et. al. 1995).

Discussion

As line transect based dung counts and faecal CMR is one of the recommended methods to assess elephant population size, there have been very few studies comparing CMR surveys with other methods. We evaluated both the methods in the given area to assess the accuracy of our population estimates, since the actual size of the surveyed population is known (approximately 34 elephants). The elephant density obtained from Line transect based dung survey is 431 per 100 sq. km. which gives us an abundance of approximately 700 elephants in the given area whereas SECR estimates were 13-38, so there is an overestimation of the numbers. The faecal-based CMR method is a more robust method than the Line transect-based dung estimation as the latter integrates estimates of the decay rate, defaecation rates, and detection rates, which are highly variable (Hedges, 2012; Kuehl et al., 2007; Plumptre, 2000). It is clear that there is growing demand for faecal DNA-based CMR studies which requires appropriate study design, adequate sample collection, genetic and statistical analysis. Our study shows that DNA-based CMR method is likely to become the method of interest for wildlife researchers and managers. The advancement can also help biologist to assess not only elephant but also assess genetic diversity, gene flow, and other parameters without any intervention (Hedges et. al, 2013). Hence, advance methodologies are likely to contribute significantly to the management and conservation of elephants and other species.

Chapter 6

MOVEMENT ECOLOGY, HOME RANGE AND HABITAT USE OF RADIO-COLLARED ELEPHANTS

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Radio-telemetry has become an indispensable tool to observe and study wild animals, especially the ones like elephant which are dangerous to be followed by foot for monitoring. Telemetry data, on such occasions can substantially add to our species knowhow by giving essential insights on home range, movement patterns, and in certain instances the breeding, dispersal and even mortality patterns of such species, all while being at a safe distance (Acharya et al. 2010; Habib et al. 2014).

As a proactive measure, the Karnataka Forest Department (KFD), Government of Karnataka had initiated an elephant telemetry programme in Kodagu and Hassan districts since 2017, to serve as an early warning system in mitigating human-elephant conflict in the region. As part of the initiative, nine female elephants (mostly identified as Matriarchs during collaring), exclusively from the herds dwelling in estates and/or responsible for major negative interactions with humans were deployed with the GPS collars in Virajpet and Madikeri divisions of Kodagu. As it provided a unique opportunity to gather much needed information on movement patterns and behavioural attributes of elephant in the landscape, permission was accorded to Wildlife Institute of India to use the existing radio telemetry data on elephants from the region, vide letter no. PCCF(WL)/E(C1)/CR-03/2016 – 17, dated 07/06/2019. Subsequently, the project

team started collecting and analysing the said data, and has been continuously monitoring the collared herds in the Kodagu district.

Methodology

Data on the elephant locations were collected from 9 female elephants fitted with GPS collars by the Karnataka Forest Department and were monitored from June, 2018 to January, 2020 (Table. 6.1). The Geotraraceur® collars were equipped with GPS transmitters that allow real time tracking and two-way communication. As Asian elephants live within a complex, matriarchal social group, only those females that were identified as matriarch were deployed with GPS collars, so as to capture the movement of an entire family group. All the elephants were collared outside the protected areas, with the objective of observing their movement and developing an early warning system to mitigate human-elephant conflict. The collars were set to download GPS fixes 5 times a day (at 0000, 0700, 1000, 1500, 1700 hrs.) to alert the resident people of any nearby elephant movement and to drive them away from human habitation. However, due to some technical problems and external factors such as canopy cover, position of the unit and mud on the collar, few collar GPS fixes were at varying time intervals. For further analysis, varying interval fixes were made uniform by post processing all the data to remove missing coordinates and multiple data points that were recorded.

A total of 10,497 GPS locations from 9 female elephants, during the period June, 2018 to January, 2020 were obtained and analysed. There were 3,469 and 7,028 locations for Dry and Wet season respectively. Locations for Irha were collected for only 53 days while for most others 200 or more days data was collected.

Minimum Convex Polygon Method and Kernel Density Estimation

Elephant home ranges (for all the locations pooled across years and seasons) were estimated using 100% Minimum Convex Polygon (MCP) and Kernel Density Estimation (KDE) using "adehabitatHR" package (Calenge, 2006) in R statistical software (R Core Team, 2019). While MCP method was used to test the comparisons of elephant home ranges from other studies, KDE is the most current and acceptable method of home range analysis in collard individuals (Walter et al., 2011). It creates a bivariate probability density function (i.e., "kernel") over each recorded point. A regular rectangular grid is then superimposed on the data and at each intersection density estimate is calculated which is essentially the average of the densities of the kernels that overlap at that point (Seaman and Powell, 1996). The utilization distribution thus generated through the KDE is sensitive to the choice of bandwidth selection (Walter et al., 2011).

We tested two KDE bandwidth estimators, namely the reference bandwidth (hereafter, href) and Least square cross validation (LSCV) to determine differences in Utilization distribution estimates of the collared elephants (Worton, 1989). KDE and LSCV for each individual were estimated at 50% and 95% isopleths contours respectively. The 50% KDE isopleths counter represent the "core" area that has the highest probability of being used by the elephants within

their home range (Worton, 1989; Ngene, 2016). The MCP and KDE (both Khref and LSCV) were estimated for all the collared female elephants for wet and dry seasons. To know the seasonal differences in the home range estimates, we used Mann-Whitney U-test using R version 4.0.3. We used ArcMap version 10.5.1 for mapping and visualizing MCP and KDE home ranges.

Individuals responding to both the resources and to each other have overlapping home ranges (Powell, 2012). To determine the mean percentage of pairwise home range overlap we used mean overlap equation, (area overlap / area of A x area of overlap / area of B) 0.5 described by Minta, 1992, where, A and B represent a pair of overlapping female elephants. We used a paired t-test to test for differences between dry and wet season for MCP and KDE.

Habitat use of radio-collared elephants

To assess the habitat-use by the collared elephant, the study area was stratified into different forest types based on the forest type map generated by the Forest Survey of India (FSI, 2014). A total of 10 forest types that fell within are identified in the study area: (1) Tropical Evergreen (TE), Semi-Evergreen (SE), Moist Teak (MT), Moist Mixed Deciduous (MMD), Dry Mixed Deciduous (DMD), Plantation (PT; including coffee, tea, pepper, cardamom, banana and coconut), Water (WT), Non-Forest (NF), Dry Deciduous Scrub (DDS) and Dry Grassland (DG).

To know the number of habitats used by an individual collared female elephant, its overall, seasonal (Dry/Wet) use of habitat along with the time of the day (Day/Night) was analysed in the ArcMap version 10.5.1. The home range (MCP) of each individual was superimposed on the study area along with the forest types and the proportion of time spent (number of locations) in each within the home range, based on season (Dry/Wet) and time of the day (Day/Night). Following the method described by Neu et al. (1974) and Byers (1984), habitat preference or avoidance for each elephant was estimated by availability (proportion of forest type within the home range) and utilization (time spent in each habitat). To estimate the expected location, the total number of locations observed was multiplied by the proportion of forest type present within the home range of each individual. A chi-square test was used to test the level of significance of the observed locations from the expected locations of each forest type. The confidence interval limit was calculated by using the formula:

$$Pi - Z\ (1-\alpha/2k)\ [pi(1-pi)/n]1/2 \leq Pi \leq Pi + Z\ (1-\alpha/2k)\ [pi(1-pi)/n]1/2$$

Where, Pi, refers to the proportion of observed location in each forest type i, Z (1-α/2k), the upper standard normal table value corresponding to the probability tail area of α/2k and k is the number of forest types. The proportional use of different forest types by elephants during day or night and in wet or dry season was normally distributed. We used paired t-test to compare the proportion of time spent in each of the forest types for, day and night values, and for dry season and wet season. All the statistical analysis was performed using R version 4.0.3.

Table 6.1 : Summary of individual elephant GPS collars in Madikeri, Virajpet and Bramagiri forest division of Kodagu, Karnataka from 2018 to 2020.

	Overall			Dry			Wet		
Name	**No. of fixes**	**Days**	**Period**	**No. of fixes**	**Days**	**Period**	**No. of fixes**	**Days**	**Period**
Aakanksha 1	1037	226	12.04.19 to 23.11.19	261	50	12.04.19 to 31.05.19	776	176	01.06.19 to 23.11.19
Aananya 1	1587	321	07.07.18 to 23.05.19	859	174	01.12.18 to 23.05.19	728	147	07.07.18 to 30.11.18
Dharni	1181	331	07.07.18 to 08.06.19	557	179	01.12.18 to 31.05.19	610	147	07.07.18 to 30.11.18
Greta	1464	226	20.05.19 to 31.11.19	281	42	20.05.19 to 31.05.19; 01.12.19 to 31.12.19	1185	183	01.06.19 to 30.11.19
Irha	167	53	08.07.18 to 04.09.18	-	-	-	167	53	08.07.18 to 04.09.18
Tamayanthi	1702	446	07.07.18 to 28.01.20	538	148	01.12.18 to 31.05.19; 06.12.19 to 28.01.20	1164	299	07.07.18 to 30.11.18; 01.06.19 to 31.10.19
Indira	1224	290	13.04.19 to 27.01.20	536	106	13.04.19 to 31.05.19; 01.12.19 to 27.01.20	812	183	01.06.19 to 30.11.19
Meera 1	1249	230	21.05.19 to 07.01.20	258	49	21.05.19 to 31.05.19; 01.12.19 to 07.01.20	991	182	01.06.19 to 30.11.19
Manimekalai	774	191	07.07.18 to 14.01.19	179	45	01.12.18 to 31.05.19; 06.12.19 to28.01.20	595	146	07.07.18 to 30.11.18

Continued monitoring of selected herds and additional collar deployments

Further to the old collars deployed by Karnataka forest department either getting their battery exhausted or experiencing technical glitches, Wildlife Institute of India in collaboration with the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH and KFD (GIZ has been strengthening the KFD by supporting pilot application of newly developed instruments for the mitigation of HWC in Karnataka through Indo-German Technical Cooperation on Human-Wildlife Conflict Mitigation in India programme), initiated re-collaring of study elephants from the region in the month of May, 2020 (Vide letter no. PCCF (WL)/C2/CR-22/2017-18, dated 02/05/2020). Subsequently different individuals (female) elephants of herd Ananya, Akanksha and Meera were radio-collared in Kodagu circle with a different id, while elephants Oldbelt, Beetamma and Bhuvaneshweri were radio-collared in Hassan circle. Additionally, collaring and translocation of conflict individuals (on pilot basis) was also initiated by the forest department,

in order to access the feasibility and success of translocation as a conflict mitigation strategy. The state currently has 4 resident females (herds) and 3 resident males collared in Kodagu, 3 resident females collared in Hassan, 2 resident males and females each collared in Nagarahole buffer and 1 resident male in MM Hills landscape. Ten problematic individuals (adult males) have been captured, collared and translocated to Male Mahadeshwara Hills – Cauvery Wildlife Sanctuary landscape (4 individuals), Bandipur Tiger Reserve (5 individuals) and Badhra TR (1 individual) since January, 2021.

GSM/Satellite based collars from African Wildlife Telemetry were used in all the collaring operations in current phase. The said collars are programmed to communicate GPS fixes every hour to an online interface. To determine home ranges (for all the locations pooled across years and seasons. The collars were programmed to download GPS fixes every hour. However, a few collar GPS fixes were at varied times due to some technical issues and circumstances like canopy cover, the positional tilt of the unit. The choice of home range estimator in spatial ecology studies can significantly influence management and conservation actions, as different methods lead to vastly different interpretations of movement patterns, habitat selection, as well as home range requirements. Quantification of home ranges is often a fundamental component in animal habitat and resource selection studies, as we can draw ecological implications across a range of spatial and temporal scales. (Silva et. al, 2018). A detailed summery of collared individuals in Karnataka are provided in Table 6.2-6.5.

Table 6.2 : Summary of collard resident elephants in Kodagu circle.

Sl. No.	Collar ID	Collar type	Elephant ID	Herd/loner	Capture Location	Release Location	Reason	Data period
1	3080	GSM	Meera 2	Herd	Virajpet	collaring location	Conflict	14-05-2020 to 24-07-2022 (still active)
2	3086 3082	GSM GSM	Akanksha 2	Herd	Virajpet	collaring location	Conflict Re-collared	18/10/2020 to 17/02/2022 17-02-2022 to 24-07-2022 (still active)
3	3087 3945	GSM Satellite	Ananya 2	Herd	Virajpet	collaring location	Conflict Re-collared	16-05-2020 to 17/02/2022 17-02-2022 to 24-07-2022 (still active)
4	3077	Satellite	Vishnu	Tusker	Virajpet	collaring location	Conflict	23-02-2022 to 24-07-2022 (still active)
5	3078	GSM	Usha	Herd	Virajpet	collaring location	Conflict	24-02-2002 to 24-07-2022 (still active)
6	3946	Satellite	Aiyappa	Tusker group	Virajpet	collaring location	Conflict	22-02-2022 to 24-07-2022 (still active)
7	3947	Satellite	Makhna	Tusker	Virajpet	collaring location	Conflict	18-02-2022 to 24-07-2022 (still active)
8	4299	Satellite	NE 2	Herd	Nagarahole buffer	collaring location	Conflict	20-08-2021 to 24-07-2022 (still active)
9	4300	Satellite	NE 4	Herd	Nagarahole buffer	collaring location	Conflict	24-08-2021 to 24-07-2022 (still active)
10	4301	Satellite	NE 3	Tusker	Nagarahole buffer	collaring location	Conflict	21-08-2021 to 24-07-2022 (still active)
11	4302	Satellite	NE 1	Tusker	Nagarahole buffer	collaring location	Conflict	19-08-2021 to 24-07-2022 (still active)

Table 6.3 : Summary of collard resident elephants in Hassan circle

Sl. No.	Collar ID	Collar type	Elephant ID	Herd/ loner	Capture Location	Release Location	Reason	Data period
1	3949	Satellite	Bhuvaneshwari	Herd	Hassan	collaring location	Conflict	28-01-2021 to 24-07-2022 (still active)
2	3953	Satellite	Beetamma	Herd	Hassan	collaring location	Translocation	22-01-2021 to 24-07-2022 (still active)
3	3951	Satellite	Old belt	Herd	Hassan	collaring location	Conflict	23-01-2021 to 22/11/2021

Table 6.4 : Summary of collared resident elephant in MM Hills.

Sl. No.	Collar ID	Collar type	Elephant ID	Herd/ loner	Capture Location	Release Location	Reason	Data period
1	4295	Satellite	Ponnachi	Tusker	MM Hills Range	collaring site	Conflict	18-07-2022 to 24-07-2022 (still active)

Table 6.5 : Summary of translocated elephants in Karnataka.

Sl. No.	Collar ID	Collar type	Elephant ID	Herd/ tusker	Capture Location	Release Location	Reason	Data period
1	3945	Satellite	Mountain	Tusker	Hassan	Cauvery WLS	Translocation	10-06-2021 to 29-06-2021
2	3948	Satellite	Chota Bheem	Tusker	Hassan	MM Hills	Translocation	27-01-2021 to 9/05/2022
3	3950	Satellite	Kusha	Tusker	Dubare	Bandipur TR	Re-wilding	03-06-2021 to 24-07-2022 (still active)
4	3952	Satellite	Gunda	Tusker	Hassan	MM Hills	Translocation	10-06-2021 to 07-12-2021
5	3954	Satellite	Colonel	Tusker	Virajpet	Bandipur TR	Translocation	11-04-2021 to 24-07-2022 (still active)
6	4297	Satellite	Haveri Tusker	Tusker	Haveri	Badhra TR	Translocation	24-02-2022 to 24-07-2022 (still active)
7	4293	Satellite	CKM Tusker	Tusker	Chikkamagalur	Bandipur TR	Translocation	09-04-2022 to 24-07-2022 (still active)
8	4294	Satellite	Basava	Tusker	Virajpet	Bandipur TR	Translocation	05-06-2022 to 24-07-2022 (still active)
9	4298	Satellite	Old Makhna	Makhna	Hassan	Bandipur TR	Translocation	30-06-2022 to 24-07-2022 (still active)
10	4296	Satellite	Matturu	Tusker	Hassan	MM Hills	Translocation	02-07-2022 to 24-07-2022 (still active)

Additionally, for the period of 2020 to 2022 we did movement analysis using more methods like Brownian bridge movement model (BBMM) as we have every hour fixes in a day. MCP and KDE (both href and LSCV) were estimated for female elephants in all four seasons namely summer, winter, north-east monsoon and south-west monsoon.

Brownian bridge movement model

The Brownian bridge movement model (BBMM), introduced by Horne et al., 2007 models an animal's movement path, rather than individual points (incorporating the distance and time lag between consecutive locations), and provides an estimate of the animal's mobility referred to

as the Brownian motion variance (σ^2 m)(Silva, et al.,2018), therefore BBMM is based on the characteristics of a conditional random walk between succeeding pairs of places, dependent on the elapsed time, the distance, and the Brownian motion variance that is associated with the animal's mobility(Horne et al, 2007). BBMM requires a time-stamped series of animal locations and the estimated telemetry error associated with each location to calculate the variance of the Brownian motion (σ^2 m) which assumes σ^2 m to be the same along the entire path (Silva et. al, 2018). BBMM can greatly enhance our understanding of home ranges, migration routes, seasonal movements, and habitat use patterns of the radio-collared elephants. The statistical analyses were done using the BBMM package of R statistical software 4.2. Home ranges were estimated for 95% and 50% levels respectively where the latter represents the core area of the elephant herds.

Movement Pattern

The relocation data were used to calculate the distance travelled by the elephants per relocation using R version 4.0.3 and MS Office (2016). Average displacement during day and night was calculated by classifying the data into day and night. We have also calculated the daily displacement in a month to see the movement pattern of elephants(males) after translocation. The fortnightly and monthly home ranges and mean daily movement in a month were plotted using MS Excel (2016) and R software. We have not included elephants with less than three months of data in the analysis. The movement pattern of the 11 radio-collared males was analysed using three parameters, mean daily movement (total displacement in a day), distance covered between two consecutive GPS locations (mean step length), and mean distance covered during day and night (temporal). We used one-way ANOVA to assess the difference in the movement parameters (total distance and mean step length) among the elephants. Multiple pairwise comparisons of two or more groups were carried out with Tukey HSD, and paired t-tests were conducted for post hoc comparisons between elephants. Movement parameters during the day and night were also compared across the individuals for the total distance covered during the period using One -Way ANOVA. All the statistical analysis was performed using R version 4.0.3 and MS Office (2016).

We also compared the movement patterns of herds of Kodagu and Hassan with NE2 (resident female of Nagarhole Tiger Reserve) and see if there is the difference as to how the movement pattern changed in comparison to the herd in the protected area. The movement pattern of the eight radio-collared (females)was analysed using two parameters, mean daily movement (total distance covered per day) and mean daily movement during day and night. All the parameters, total distance (between individuals), and mean distance between day and night (between individuals) were compared, using one-way ANOVA to assess the difference in the movement parameters (total distance) among the elephants. Multiple pairwise comparisons were carried out with Tukey HSD and paired t-tests were conducted for post hoc comparison between the groups. Movement parameters during the day and night were also compared across the individuals for the total distance and all the statistical analyses were performed using R version 4.2.

Habitat Availability and Utilisation

Habitat selection by wildlife is an important aspect of ecology. Studies of habitat selection provide information on environmental characteristics needed by animals, essential knowledge for the development of wildlife management, and conservation policies. The habitat of an organism is often described by only categorical variable (vegetation type), and the study of habitat selection by a species often consists of the identification of the preferred or avoided habitat types (Neu et al. 1974). This is mainly carried out by comparing the availability of each habitat type to the use of that habitat type by the species. The study of habitat selection by an organism is generally carried out by comparing its habitat use to habitat availability. According to Thomas and Taylor's (1990) distinction of three kinds of designs, according to which the levels of use and availability are measured. With designs of type I, the individual animals are not identified, and both use and availability are measured at the scale of the population. Designs of type II rely on identified individuals and the use is measured for each one, but availability is still measured at the scale of the population (study of the distribution of animal's home ranges use on a study area) and type III design also rely on identified individual animals, but both use and availability are measured at the scale of the individual. Here our aim of the analysis is to identify the variables affecting site selection by animals within their home range and therefore we are using type III design to calculate the use and availability of their home range area. The "availability" of land-use categories was calculated within the 100% MCP home range and the "used" area is calculated from 95% LSCV as it deems to be a better-fitted model. The analysis was done in R statistical software 4.2 using the adehabitatHS package. To further determine the preference or avoidance of habitats by elephants, we have used the Manly selection ratios (Manly, McDonald, Thomas, McDonald, & Erickson, 2003) by comparing the observed number of elephant locations in each habitat type to those we expect based on the proportion of each habitat type available. Significance of selectivity ratio (wi) was determined using loglikelihood and 95% confidence intervals for each habitat category; a selectivity ratio >1 indicates disproportionate preference, and values.

Results

6.1 Ranging pattern of radio-collared elephants from 2018-2020

All the collared females range outside the protected area in the human-dominated landscape, except Aananya 1 and Greta, whose 30% and 15% area of total 100% MCP falls within the protected area (Figure 6.1). Home range estimates of the collared elephants varied greatly between different estimators (MCP and KDE bandwidths) and contours. Mean home range estimates of 100% MCP were greater than the KDE estimates (both href and LSCV). However, due to the small sample size for Irha and Manimekalai, the 95% contour of href was greater than the 100% MCP, which may be due to over-smoothing by href bandwidth at 95% contour. The core area ranges at 50% contour for href and LSCV varied greatly among the collared elephants. A detailed description of results is tabulated in Table 6.6.

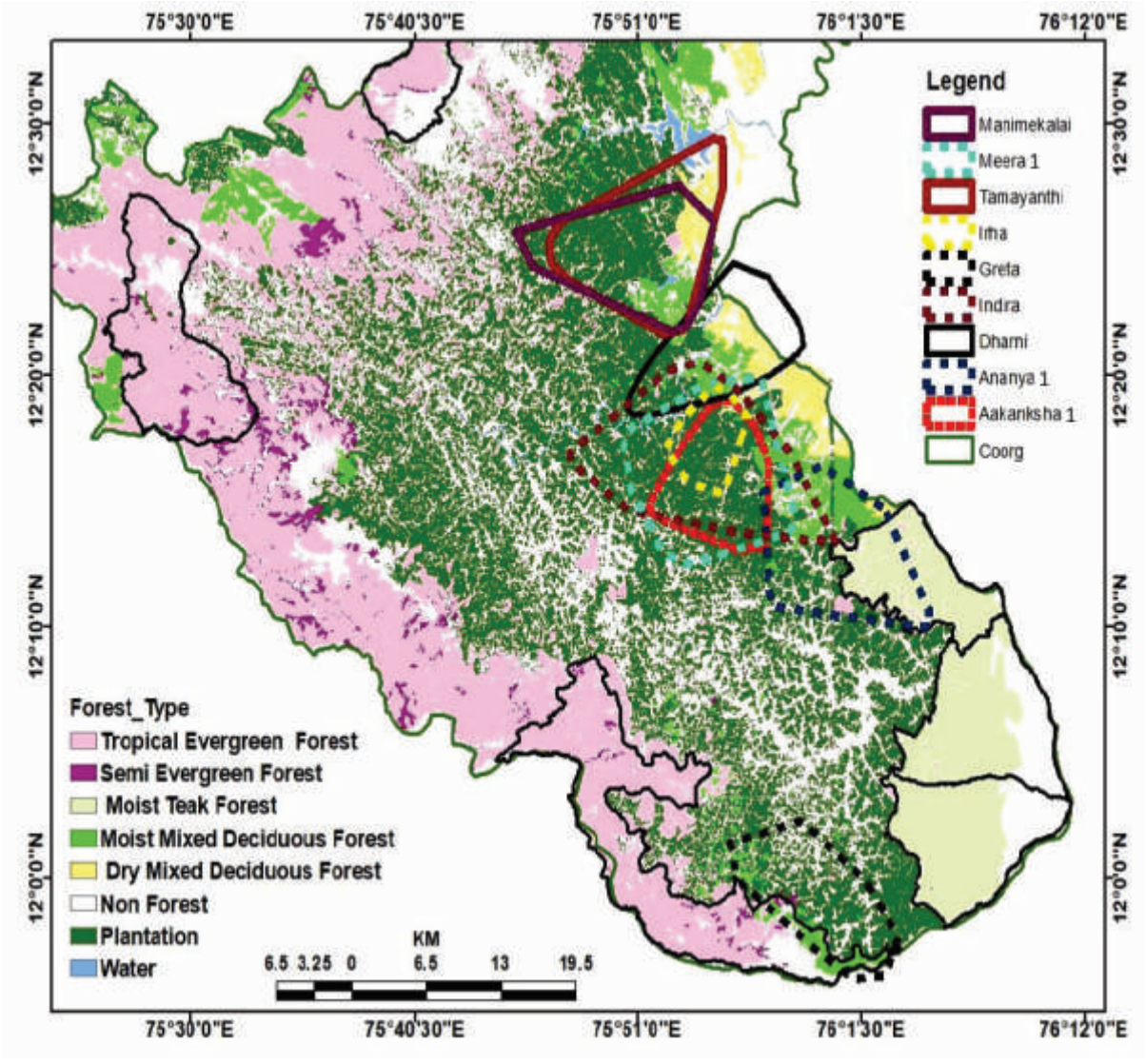


Figure 6.1 : *Comparison of home range area (100% MCP) for radio-collared elephants in 2018-2019*

Table 6.6 : Seasonal home ranges of nine collared elephants as determined by MCP and KDE analysis

	Overall					Dry					Wet				
Name	MCP (100%)	href (50%)	href (95%)	LSCV (50%)	LSCV (95%)	MCP (100%)	href (50%)	href (95%)	LSCV (50%)	LSCV (95%)	MCP (100%)	href (50%)	href (95%)	LSCV (50%)	LSCV (95%)
Aakanksha 1	74.53	13.26	52.36	3.57	16.79	53.55	12.25	48.59	2.65	12.74	52.25	13.42	52.44	3.76	16.78
Aanaya 1	113.77	17.82	76.22	5.00	24.36	73.30	16.54	69.08	4.07	20.61	93.42	21.27	88.24	5.11	21.41
Dharni	91.14	22.55	92.70	7.65	35.94	68.42	25.57	85.84	6.63	28.14	54.13	07.45	39.41	3.48	18.20
Greta	87.98	19.57	80.43	4.12	24.00	44.60	18.50	68.38	1.82	09.62	87.98	18.17	77.64	3.71	21.55
Irha	33.14	10.44	46.65	2.92	14.03	-	-	-	-	-	33.14	10.44	46.66	2.92	14.03
Tamayanthi	123.97	27.07	113.25	7.56	34.75	118.82	40.25	160.19	7.08	32.43	59.15	23.99	79.98	5.61	25.45
Indira	169.42	48.21	163.72	7.96	45.94	147.21	26.90	105.70	4.67	23.75	142.3	51.31	183.63	7.24	42.75
Meera 1	143.61	35.04	138.23	5.98	36.37	58.89	11.17	67.65	2.17	14.53	143.61	39.48	141.75	6.48	34.96
Manimekalai	109.11	30.69	120.97	7.04	31.72	44.77	16.48	67.17	4.78	19.38	106.47	36.61	128.22	6.71	31.39
Mean	105.19	24.96	98.28	5.76	29.32	76.20	20.96	84.08	4.23	20.15	85.83	24.68	93.11	5.00	25.17
SD	39.79	11.8	39.10	1.92	10.30	37.28	09.61	34.95	1.96	07.80	39.74	14.80	48.64	1.60	9.45

Table 6.7 : Mean percentage pairwise home range overlap in overall, dry and wet season for MCP and KDE analysis at 95% and 50% isopleths respectively.

Percentage overlap (±SE)						
Home Range						
	Overall	**n**	**Dry**	**n**	**Wet**	**n**
MCP (100%)	32.17 ± 7.99	15	36.13 ± 10.38	8	41.72 ± 9.11	10
href (95%)	35.18 ± 7.95	13	24.35 ± 8.12	9	35.69 ± 8.12	12
LSCV (95%)	19.25 ± 5.15	12	12.07 ± 5.05	7	20.32 ± 5.48	11
Core area						
LSCV (50%)	14.97 ± 4.03	7	8.75 ± 5.79	5	11.48 ± 2.97	8
href (50%)	27.54 ± 4.95	6	37.97	1	30.38 ± 6.11	6

Table 6.8 : Summary of forest type preference by collared elephants of Kodagu in dry season

	Tropical evergreen			**Semi-evergreen**			**Moist-Teak**			**Moist mixed deciduous**			**Dry mixed deciduous**			**Plantation**			**Water**			**Non forest**			**Dry deciduous scrub**			**Dry grassland**		
	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N
Aakanksha1	NS	NS	NS	/	/	/	/	/	/	NS	NS	NS	/	/	/	NS	NS	NS	/	/	/	NS	NS	NS	/	/	/	/	/	/
Aananya1	-	-		/	/	/	+	+		+	+	+	-	-	-	-	-	-	/	/	/	-	-		/	/	/	/	/	/
Indira				/	/	/		/		+	+	+	+	+	+	-	-	-	-	-	-		-	-	/	/	/	/	/	/
Meera1		/	NS	/	/	/	/	/	/	+	+	NS			NS	-	-	NS	/	/	/			NS	/	/	/	/	/	/
Tamayanthi	-			/	/	/	/	/	/	+	+					-	-		-	-	/							/	/	/
Dharni			NS	/	/	/	/	/	/	+	+	NS			NS			NS			NS	-	-	NS	/	/	/	/	/	/
Greta			NS			NS	/	/	/	+	+	NS	/	/	/			NS	/	/	NS	-	-	NS	/	/	/			NS
Manimekalai		-	NS	/	/	/	/	/	/	+	+	NS	+	+	NS	-	-	NS	/	/	/			NS	/	/	/	/	/	/

Table 6.9 : Summary of forest type preference by collared elephants of Kodagu in wet season.

	Tropical evergreen			**Semi-evergreen**			**Moist-Teak**			**Moist mixed deciduous**			**Dry mixed deciduous**			**Plantation**			**Water**			**Non forest**			**Dry deciduous scrub**			**Dry grassland**		
	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N	T	D	N
Aakanksha1	/	/	/	/	/	/	/	/	/	-		/				+	+		/	/	/		-		/	/	/	/	/	/
Aananya1				/	/	/			-	+	+	+	/	/	/	-	-	-	/	/	/	-	-		/	/	/	/	/	/
Indira				/	/	/	/	/	/	+	+	+				-	-	-	-	/	-		-	+	/	/	/	/	/	/
Irha			NS	/	/	/	/	/	/	+	+	NS			NS	-	-	NS	/	/	/			NS	/	/	/	/	/	/
Meera1	+	+		/	/	/	/	/	/	+	+	+				-	-		/	/	/	-	-		/	/	/	/	/	/
Tamayanthi				/	/	/	/	/	/	+	+					-	-	-		-					/	/	/	/	/	/
Dharni	/	/	/	/	/	/	/	/	/	-	-	-	+	+	+	/	/	/	/	/	/	-	-			/		/	/	/
Greta	+	+		-	-	-	/	/	/	+	+		/	/	/				/	/	/	-	-	-	/	/	/			
Manimekalai	-	-		/	/	/	/	/	/	+	+	+	+	+		-	-	-							/	/	/	/	/	

"/" = No location present; "NS": Not significant; "+" = Forest type is used more than expected; "- "= Forest type is used less than expected; Blank cells = forest type is used in proportion to availability. T= total, D= Day, N= Night

Habitat use of radio-collared elephants

There was significant difference in overall use of forest types by collared elephants ($\chi2=2382.47$; d.f. = 9; $p<0.001$). The intensity of use of different forest types varied among the individuals (Table 6.8 & 6.9). For instance, the plantation was used most intensively by Aakanksha 1 (84%), followed by Irha (60%), Indira (52%), Meera 1 (59%), Greta (52%), and Tamayanthi (45%), whereas moist mixed deciduous forest was used more intensively by Aananya 1 (45%), while Dharni (54%) used dry mixed deciduous forest more intensively than the other available forest types.The confidence interval showed that most of the collared

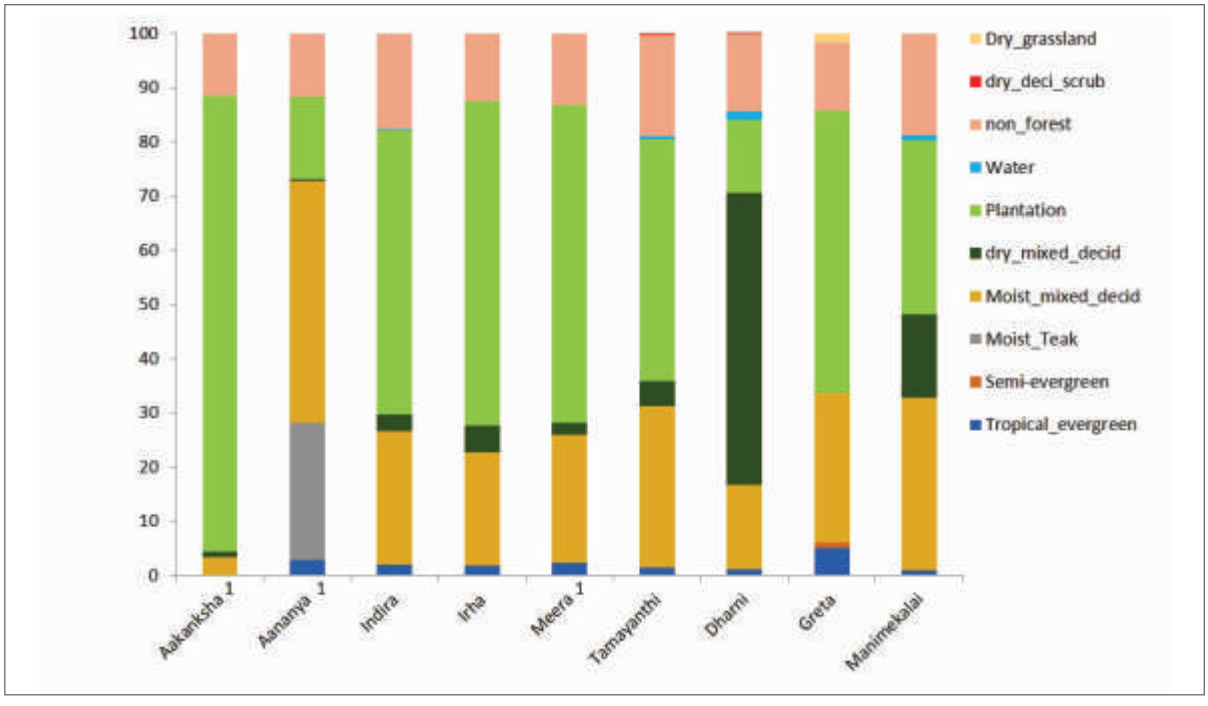


Figure 6.2 : *Proportion of forest types used within the home range of collared elephants.*

elephants (70%) preferred the moist mixed deciduous forest more in proportion to the availability. Although plantation was used more intensively by most female elephants, however the plantation was used less than expected when the use is compared with the proportion of availability. Non-forest areas were used less than expected by 44% of the collared females, while rest of the females used the non-forest areas in proportion to the availability. Dry mixed deciduous forest was preferred by Indira, Dharni and Manimekalai, whereas Aananya 1 and Tamayanthi tend to use less. Aakanksha 1, Irha and Meera 1 used dry mixed deciduous forest in proportion to the availability. Tropical evergreen forest was used less by Aananya 1, Dharni and Manimekalai, but preferred by Greta and used in proportion to the availability by other elephants.

The forest types used by the elephants do not differ significantly during the day and night time ($t = -0.017$, d.f. = 5, $p = 0.49$) except for non-forest area for which the intensity of use was more during the night (23%, n = 604), than during the day (13%, n = 931). However, there was a significant difference between the use of forest type and to the availability during day and night. During the day time only Aakanksha 1 preferred the plantation while the rest of the elephants used less than expected. Most elephants used less than expected non-forest area during the day time, but the same was used more than expected by Indira during the night time and used in the proportion to the availability by the other elephants during the night time. In the other forest types, no difference was observed in the selection of forest types during day and night.

6.2 Ranging Pattern of resident radio-collared elephants from 2020-2022

Home range estimation

Home range estimates of the collared elephants varied greatly between different estimators (MCP and KDE bandwidths and contours and BBMM analysis) Mean home range estimates of 100% MCP were greater than the KDE estimates (both href and LSCV). However, due to the small sample size for Makhna and Usha, the 99% contour of href was greater than the 100% MCP, which may be due to over-smoothing by href bandwidth at 99% contour. The core area ranges at 50% contour for href and LSCV varied greatly among the collared elephants. A detailed description of results is tabulated in sq. km in the following table 6.10.

Table 6.10 : Overall home ranges for radio-collared individuals by MCP, KDE and BBMM analysis in km²

Individual	Data period	No of Fixes	No of days	MCP 100 %	MCP 95%	MCP 50%	KD href 99%	KD href 95%	KD href 50 %	KD LSCV 99%	KD LSCV 95%	KD LSCV 50%	BBMM 95%	BBMM 50%
Meera 2	14/05/2020 to 19/02/2022, 20/03/2022 to 10/04/2022	15562	669	273.178	253.538	60.206	264.845	190.500	46.845	120.224	87.743	15.762	105.647	17.889
Akansha 2	18/10/2020 to 17/02/2022	11934	488	127.488	72.456	13.282	92.801	56.275	11.223	37.575	25.881	4.215	31.923	4.757
Ananya 2	16/05/2020 to 17/02/2022	14881	643	116.677	86.524	29.973	110.019	81.850	19.014	52.790	37.215	6.434	36.613	7.309
Usha	24/02/2022 to 12/10/2022	5453	231	143.089	119.200	37.490	164.069	122.360	27.130	59.980	44.150	8.230	45.643	7.523
Aiyappa	22/02/2022 to 12/10/2022	4640	233	139.040	98.480	14.370	120.430	83.320	12.840	37.510	26.680	3.530	30.577	3.899
Makhna	19/02/2022 to 13/10/2022	4973	237	142.070	112.210	18.580	143.720	96.940	16.250	43.100	28.010	3.960	26.802	3.495
Beetamma	23/01/2021 to 10/04/2022	9397	443	529.269	311.867	53.741	395.154	260.685	49.313	133.460	93.219	15.733	93.310	14.388
Bhuveneshwari	29/01/2021 to 10/04/2022	9512	437	583.404	546.845	74.988	619.569	426.959	81.460	194.277	128.138	18.866	85.626	11.807
Oldbelt	24/01/2021 to 22/11/2021	6553	303	242.782	208.946	59.334	300.157	221.131	42.719	99.619	70.146	12.067	74.594	10.208
NE2	21/08/2021 to 12/10/2022	10164	418	296.370	232.390	50.150	277.460	183.710	33.670	128.090	91.240	17.340	89.055	15.677
NE4	25/08/2021 to 12/10/2022	11695	414	184.620	151.560	56.240	185.510	139.070	36.070	80.470	59.780	12.010	52.242	9.152

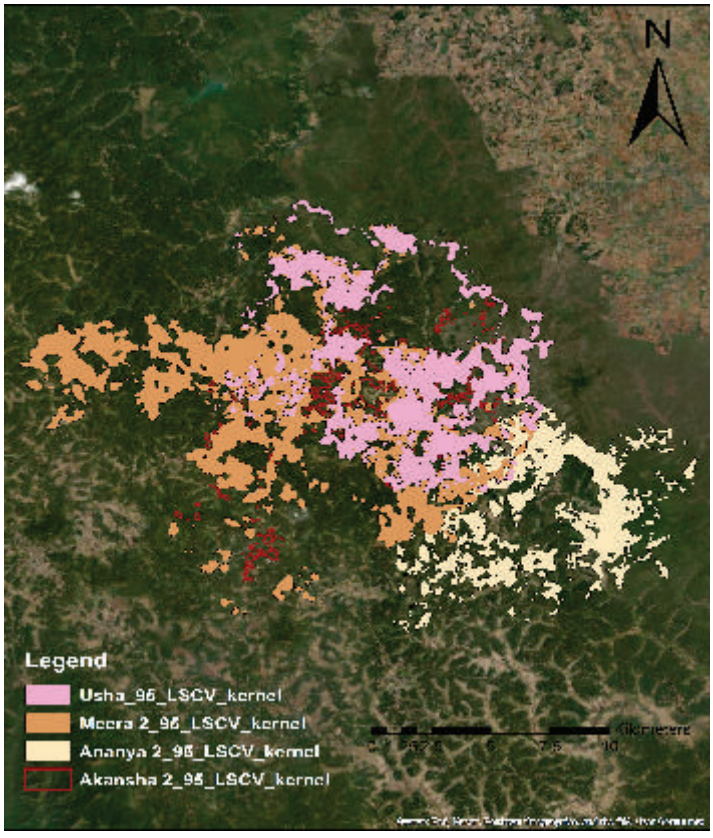


Figure 6.3 : *Home range of female individuals of Kodagu (Kernel Density estimation)*

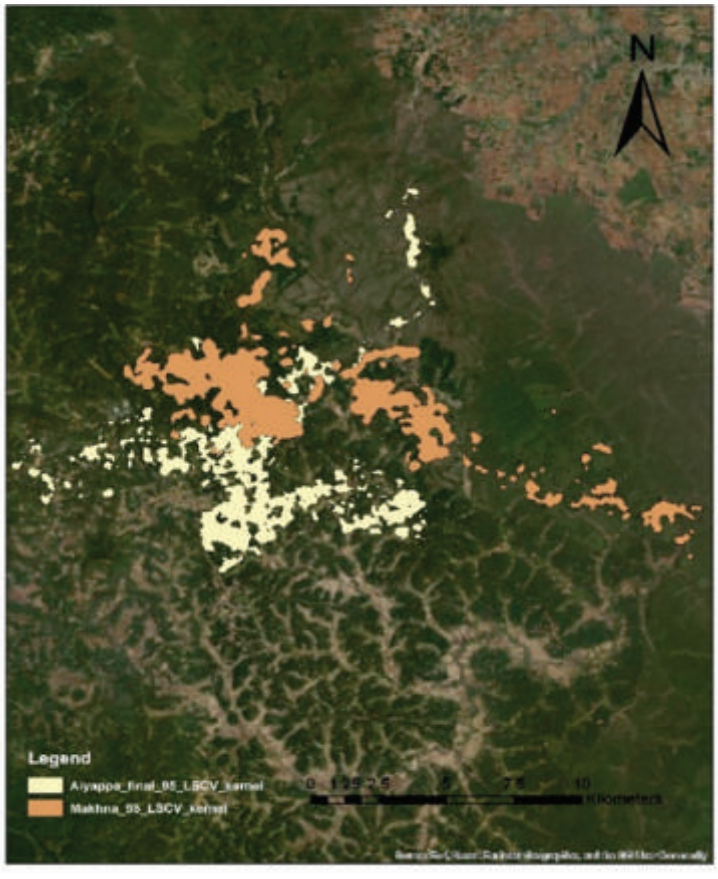


Figure 6.4: *Home range of male individuals of Kodagu (Kernel Density estimation)*

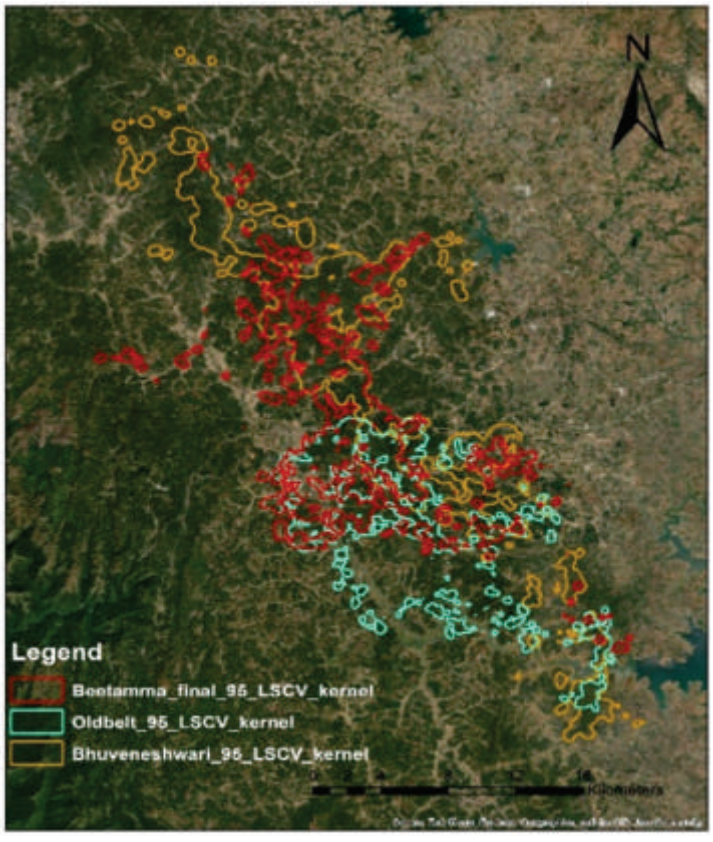


Figure 6.5 : *Home range of Hassan individuals (Kernel Density Estimation)*

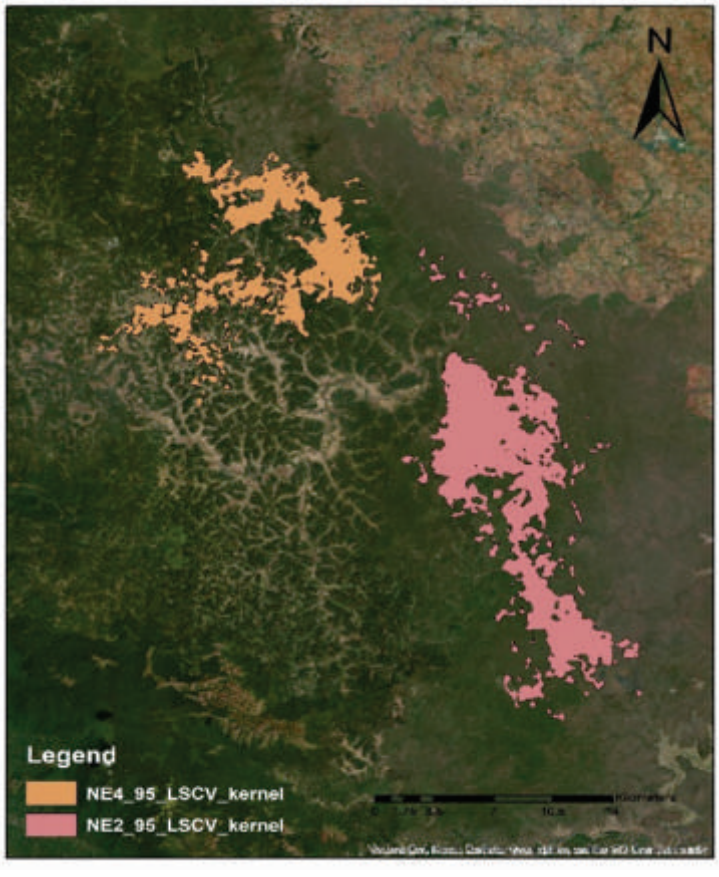


Figure 6.6 : *Home range of Nagarhole individuals (Kernel Density Estimation)*

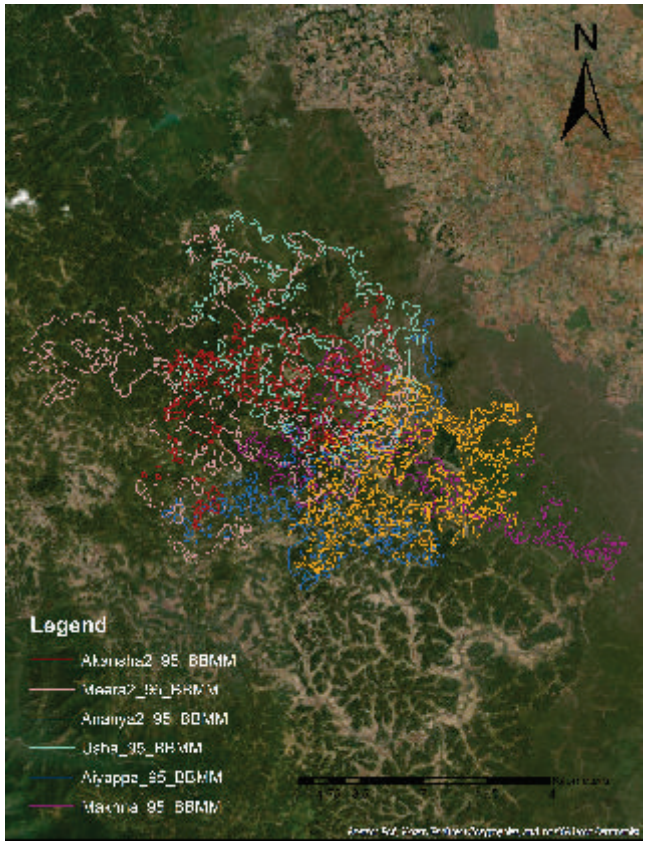


Figure 6.7 : *Home range of Kodagu individuals (Brownian bridge movement model)*

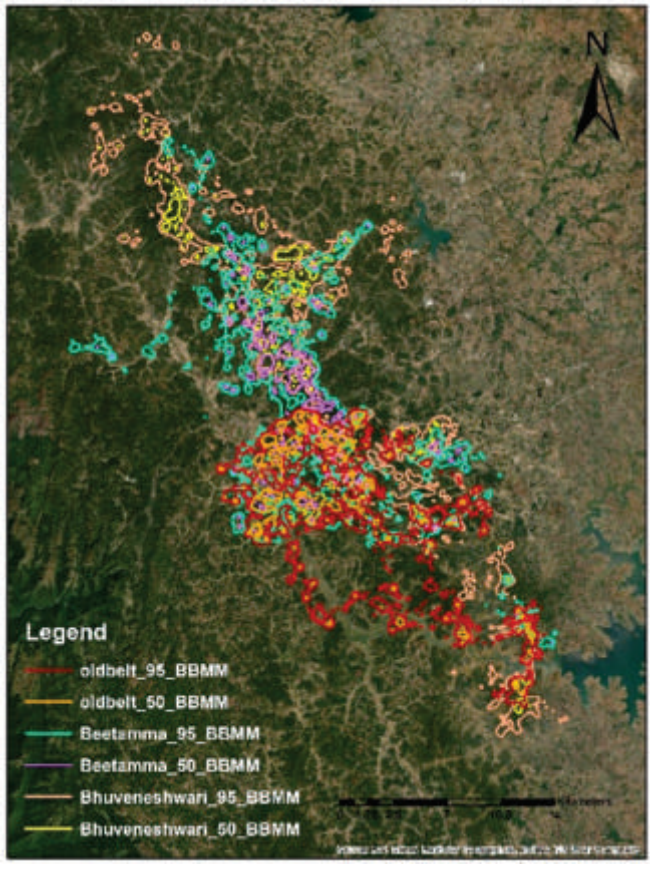


Figure 6.8 : *Home range of Hassan individuals (Brownian bridge movement model)*

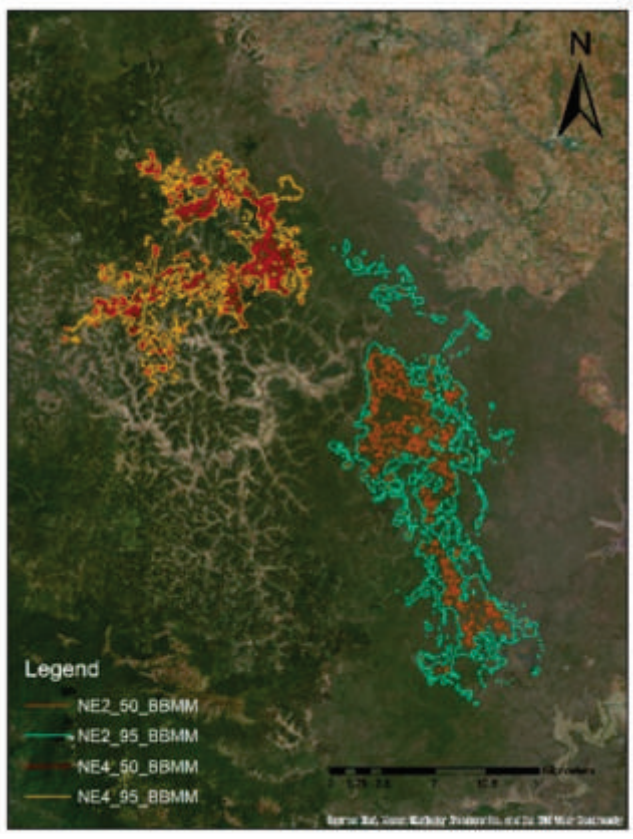


Figure 6.9 : *Home range of NTR individuals (Brownian bridge movement model)*

Overlapping in Home Ranges of Herds

Elephants radio-collared in coffee estates of Kodagu

The collared females radio-collared in the Virajpet area mostly inhabit the coffee plantation area and it is seen that there is overlap among these herds. Overlapping areas were calculated from 100% MCP. Meera 2 and Akansha 2 have an overlapping area of 127.48 sq. km (99.93%), Meera 2 and Ananya 2 are 31.07 sq. km(11.37%), whereas Ananya 2 and Akansha 2 have an overlapping area of 11.26 sq. km(8.83%). Ananya is the only herd whose home range coincides with the protected area of Nagarhole Tiger Reserve with an overlapping area of 24.96sq. km (21.34%). Both Meera 2 and Akansha 2 however combine and splits up occasionally depending on the availability of resources. Usha has an overlap with Meera 2 of 131.93 sq. km (48.29 %), Akansha 2 of 83.74 sq. km (65.69%), and Ananya 2 of 19.72 sq. km(16.90%). Usha also has a coinciding area of 28.06 sq. km (19.61%). Similarly male residents Aiyappa and Makhna share territory with each of the female herds. Aiyappa has an overlapping area with Meera 2 of 72.28 sq. km (51.98%), Ananya 2, 80.47 sq. km (57.87%), Akansha2, 37.54 sq. km (27%), and Usha 31.9 sq. km (23%), whereas Makhna has an overlapping area with Meera 2, 56.70 sq. km (39.91%), Ananya 2, 87.84 sq. km (61.83%), Akansha 2, 36.02 sq. km (25.35%) and Usha 37.42 sq. km (26.34%). Even Aiyappa and Makhna intersect an area of 75.01 sq. km (53.95%). Makhna also ventures into the NTR area with an overlap of 48.86sq. km (34.32%).

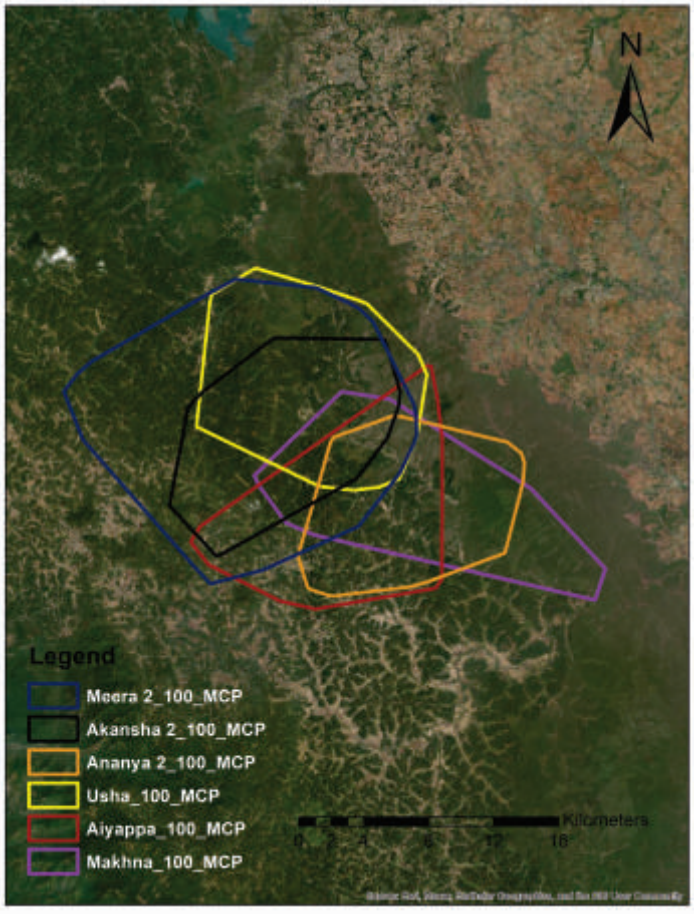


Figure 6.10 : *Home range overlap between 100% MCP of Kodagu individuals*

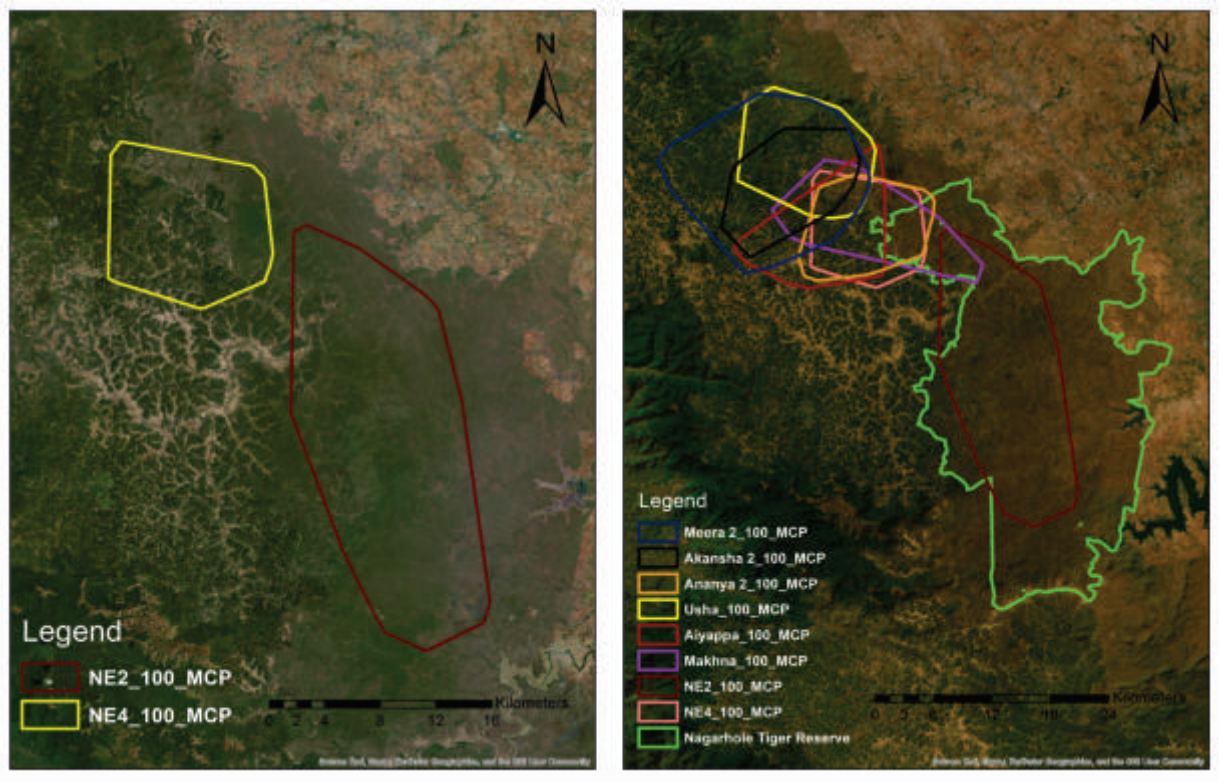


Figure 6.11 : *Home range overlap between 100% MCP of NTR with Kodagu individuals*

Elephants radio-collared in Nagarhole Tiger Reserve

Similarly, the two herds namely NE2 and NE4 radio-collared within the protected area of Nagarhole Tiger Reserve. The area of NE4 shows that the herd strays away from the protected area boundary to the coffee plantations of Kodagu.

The home range area overlaps of NE4 with the Nagarhole protected area is 22.56 sq. km (12.19%) calculated from 100%MCP. NE2 stays mostly within the protected area boundaries. NE4 has an overlapping area with all the herds of Kodagu and also the male residents Aiyappa and Makhna. NE4 has an overlapping area of 22.01 sq. km (11.92%) with Akansha 2, 111.56 sq. km (60.43%) with Ananya 2, 48.23 sq. km (26.12%) with Meera 2, 28.06 sq. km (15.20%) with Usha, highest being with the herd Ananya 2. Even NE4 shares territory with Makhna 100.64sq. km (54.51%) and Aiyappa 107.38sq. km (58.16%).

Elephants radio-collared in Hasaan

The three herds were radio-collared in Hassan which mainly consists of fragmented patches, coffee plantations and agricultural fields lying mostly in the human-dominated landscape. The three herds Beetamma, Bhuveneshwari and Oldbelt herd overlaps with each other. From the home range calculated from 100%MCP, Beetamma and Bhuveneshwari have an overlapping area of 411.63 sq. km (77.77%) whereas Beetamma and Olbelt have an overlapping area of 175.22sq. km(33.10%). Bhuveneshwari shares an overlapping area of 124.80 Sq. km (29.96%) with oldbelt herd.

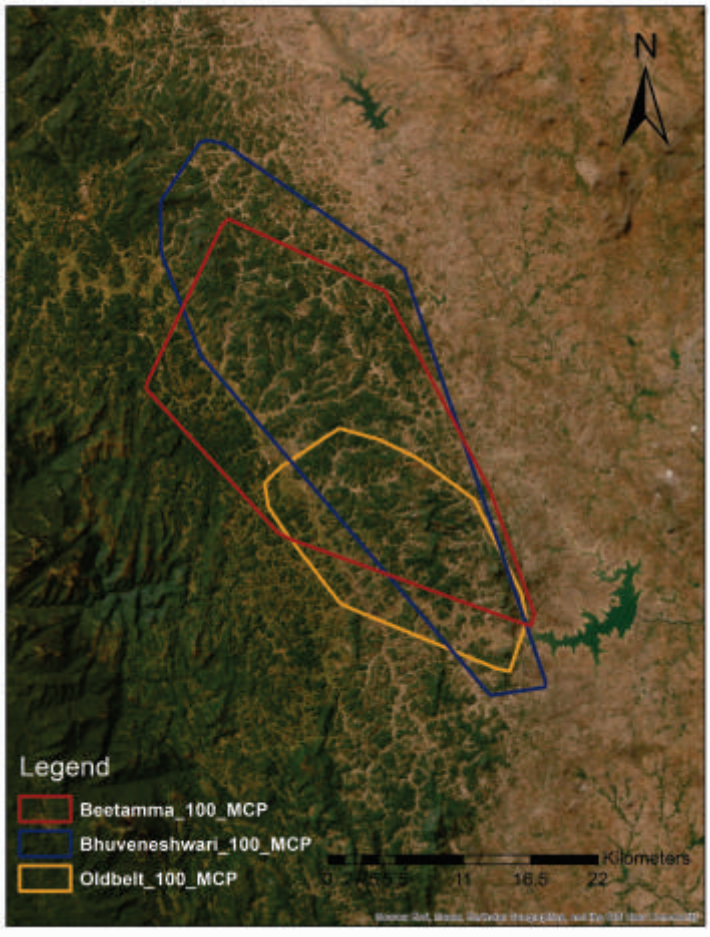


Figure 6.12 : *Home range overlap between 100% MCP of Hassan individuals*

Seasonal home range estimation

Similarly, seasonal variation among home range estimates was also calculated by dividing it into four seasons. The three study areas Virajpet, Hassan, and Nagarhole Tiger Reserve more or less have the same seasons with a variation of rainfall of 15 days. A year was divided into four seasons namely Winter (January-February), Summer (March-May), South West Monsoon (June-September), and North East Monsoon (October-December). South West Monsoon period has the highest rainfall than North-West Monsoon. Most of the crops like that of paddy, maize, and pulses are grown perennially with proper irrigation channels. Rice of different breeds is grown rotation wise one is in the Kharif season (June-September), (Sridhara et al, 2019) which is also the southwest monsoon season, and the rabi season (October to March). The horticultural crops like Coffee mostly plantations that occupy Kodagu and Hassan districts to a larger extent planted from June to December, and harvested around October to December. Paddy and coffee are the most affected crops due to elephant raids which often lead to human-elephant conflict. The dry season is considered from December to May whereas the wet season is from June to November. From the home range estimates (MCP, KDE) it is seen that there is a difference in the area due to seasonal differences.

The home range estimates from KDE LSCV 95 isopleth contours inferred that elephant herds of Kodagu mainly that of Meera 2, Ananya 2, Usha, and Makhna are greater during the South West

Monsoon. Aiyappa however shares an almost equal home range during summer and southwest monsoon seasons. Akansha home range estimates were greater in the summer season followed by the Northeast monsoon season. NE2 and NE4 radio-collared in Nagarhole Tiger Reserve have larger estimates of home range during Northeast Monsoon and Southwest Monsoon respectively. Bhuveneshwari and Oldbelt of Hassan have a larger area of home range during the winter season followed by the Northeast season whereas Beetamma has a larger home range estimate during the southwest monsoon period followed by the Northeast monsoon. A detailed description of results in sq. km is tabulated in the following table 6.11.

Table 6.11 : Seasonal Home range for resident radio-collared individuals

Elephant	Summer			Winter			SW_Monsoon			NE_Monsoon		
	MCP			MCP			MCP			MCP		
	100	95	50	100	95	50	100	95	50	100	95	50
Meera 2	135.13	99.23	50.87	74.4	65.47	10.49	161.67	134.3	39.28	193.84	176.53	30.44
Akansha 2	42.63	35.36	6.56	12.62	7.82	2.29	47.52	33.27	7.97	105.9	87.27	10.67
Ananya 2	73.89	61.14	20.66	70.03	61.24	16.84	95.65	81.05	22.17	73.69	56.9	15.32
Beetamma	139.43	111.82	34.69	239.13	167.74	18.43	193.78	147.29	45.72	191.31	116.57	36.58
Bhuveneshwari	200.15	108.60	36.90	241.03	223.82	141.02	191.00	145.30	41.36	196.86	183.40	35.15
OldBelt	126.11	114.23	47.17	242.81	208.99	59.35	75.51	63.92	30.77	101.80	93.40	38.90
NE2	153.56	141.60	84.29	181.85	164.57	44.28	128.63	67.44	16.31	226.15	205.06	56.04
NE4	100.86	89.89	40.05	56.44	49.85	22.96	167.29	134.70	82.41	80.01	66.90	15.28
Usha	103.28	72.42	17.27	12.52	12.40	6.83	134.28	107.57	55.45	6.93	6.54	1.87
Makhna	45.94	28.27	7.61	7.99	7.91	6.25	77.09	49.30	16.06	31.47	22.74	6.69
Aiyappa	88.00	75.22	13.54	1.09	0.97	0.16	87.26	69.71	16.54	NA	NA	NA
Elephant	**KDE (href)**			**KDE (href)**			**KDE (href)**			**KDE (href)**		
	99	**95**	**50**	**99**	**95**	**50**	**99**	**95**	**50**	**99**	**95**	**50**
Meera 2	174.7	130.6	31.08	88.11	63.45	12.66	173.62	124.77	32.04	222.88	165.89	36.88
Akansha 2	15.65	11.36	1.81	12.6	7.77	1.57	44.02	31.15	7.38	102.82	67.9	11.09
Ananya 2	91.45	66.63	17.44	86.77	61.09	14.13	103.36	77.77	17.11	77.35	54.77	10.91
Beetamma	162.29	118.36	30.84	268.52	183.00	32.03	223.15	155.34	32.11	249.79	173.70	37.86
Bhuveneshwari	217.96	147.68	27.68	717.13	515.95	127.54	209.18	145.87	28.33	296.14	213.28	50.44
OldBelt	183.23	139.66	34.35	300.15	221.31	42.71	99.34	77.19	23.53	180.56	135.27	36.06
NE2	266.22	184.12	36.86	342.72	244.21	54.02	131.83	86.28	16.00	282.28	198.42	41.52
NE4	137.96	105.63	27.42	81.38	59.52	17.55	206.37	156.98	39.73	85.97	62.19	14.56
Usha	109.19	75.27	15.37	37.84	27.77	7.74	171.80	130.80	32.67	14.08	10.46	3.10
Makhna	44.04	27.29	5.26	NA	NA	NA	89.82	60.14	11.42	56.19	39.96	8.60
Aiyappa	97.52	69.67	15.00	1.99	1.45	0.26	97.76	68.92	13.13	NA	NA	NA
Elephant	**KDE (LSCV)**			**KDE (LSCV)**			**KDE (LSCV)**			**KDE (LSCV)**		
	99	**95**	**50**	**99**	**95**	**50**	**99**	**95**	**50**	**99**	**95**	**50**
Meera 2	57.79	41.87	7.06	25.79	18.39	3.4	65.55	48.28	9.45	68.32	48.61	8.54
Akansha 2	49.86	35.5	6.02	4.12	3.11	0.7	17.19	12.87	2.7	28.59	19.86	3.44
Ananya 2	28.62	20.27	3.17	20.98	15.4	3.16	40.53	29.55	4.79	25.68	18.75	3.54
Beetamma	50.24	37.13	7.68	54.75	36.87	5.93	58.91	42.46	7.55	57.00	40.38	7.39
Bhuveneshwari	59.30	41.41	7.31	124.51	85.72	12.75	49.16	33.54	5.00	63.85	44.27	7.30
OldBelt	41.99	29.85	5.36	99.63	70.15	12.07	35.68	26.10	4.81	39.61	27.89	4.49
NE2	63.76	44.99	9.96	82.79	60.19	10.73	48.67	36.64	7.74	90.30	66.54	13.18
NE4	41.10	30.54	5.12	22.17	16.72	3.80	69.45	51.48	9.58	29.10	21.61	4.45
Usha	30.44	22.04	3.94	4.03	2.87	0.64	48.49	34.71	6.95	2.69	1.99	0.48
Makhna	11.38	8.15	1.33	NA	NA	NA	19.97	13.73	2.18	10.15	7.18	1.33
Aiyappa	27.64	20.23	3.00	0.77	0.54	0.09	26.63	18.86	2.80	NA	NA	NA

From the seasonal variation of the home range (100% MCP) that of Kodagu herds it was seen that the home range of Akansha 2 integrates fully with that of Meera 2 during the Southwest monsoon, Northeast monsoon, and winter and during the summer season there is almost no change. Meera 2 and Ananya 2 have very little overlap during the Southwest monsoon (1.5 sq. km, 1.59%). In Summer season the overlap among these two are 13.49 sq. km(18.26%). Meera 2 and Usha have a high overlap during Southwest monsoon season (80.58 sq. km, 49.83%) but their home ranges almost completely integrate during winter. Aiyappa the male resident of Virajpet area has the highest overlap during southwest monsoon with Ananya 2 (68.94 sq. km,72.05%) whereas in summer (40.18 sq. km, 45.66%). Makhna also has the highest overlap with Ananya 2 during southwest monsoon season (46.22 sq. km, 59.96%). One of the herds radio-collared in Nagarhole Tiger Reserve mainly strays to plantations of Kodagu area and has an overlap with Ananya 2 during Southwest monsoon (84.56 sq. km,50.54%) and highest being in winter (52.19 sq. km, 92.45%). Makhna one of the tuskless male residents of Virajpet area also coincides with NE4 with the highest overlap in Southwest monsoon (65.63 sq. km, 39.23%), followed by summer (59.81 sq. km, 5.9%) with the least overlap during winters. NE2 stays mostly within the Nagarhole Tiger Reserve so there is no overlap with NE2. Beetamma and Bhuveneshwari of Hassan have the highest overlap in the winter season (144.24 sq. km, 59.84%) and the least in Northeast monsoon (63.48 sq. km, 32.24%). Bhuveneshwari and Oldbelt have the highest overlap in their home range during winter (144.176 sq. km, 59.37%) and northeast monsoon (51.29 sq. km, 26.05%). But during Southwest monsoon, they are coinciding less and during summer their home range gets completely separated. Similarly, Beetamma and Oldbelt have the highest overlap in home range during winters (79.77 sq. km, 32.85%) followed by in summer (17.34 sq. km, 13.75%), but in northeast monsoon, Oldbelt's homerange gets completely integrated into the home range of Beetamma.

Sinuosity Index

The sinuosity index as defined by Benhamou (2004) is an appropriate measure of the tortuosity of a random search path. Sinuosity is a function of the mean cosine of turning angles and is a corrected form of the original sinuosity index defined by Bovet & Benhamou (1988). It identifies different phases of the path, and their path tortuosity is a dimensionless feature of the path, depending mostly on the path shape, not on the unit of measurement. The tortuosity of oriented paths is linked to the efficiency of the orientation mechanism, the more the animal is efficient, the more its path should be close to the straight-line segment linking the starting point to the goal (Benhamou, 2003).

The most common characteristic used to describe and analyse movement paths is their tortuosity, or how tortuous and twisted a path is in a given space or time (Codling et al. 2008). Tortuosity has been inferred by a variety of movement measures that frequently express different information or concepts about movement behaviour (Benhamou et al; 2004). Sinuosity Index is calculated by:-

$$\text{Sinousity Index (SI)} = 2[p\{(1-c^2-s^2/(1-c)^2+s^2\}+b^2]^{-0.5}$$

Where p = mean step length, c = mean cosine of turning angles, s = mean sine of turning angles, and b = coefficient of variation of step length (Bovet & Benhamou;1988, Benhamou;2004). Here we used radio telemetry data of eleven individuals of different landscapes and divided the data by seasons. The Sinuosity Index and Emax are found by using trajr R package in R statistical software 4.3, where Emax or E^a max is a dimensionless estimate of the maximum expected displacement of a trajectory. Larger Emax values (approaching infinity) represent straighter paths (Cheung, Zhang, Stricker, & Srinivasan, 2007). E^b max is E^a max multiplied by the mean step length, so gives the maximum possible displacement in spatial units.

In the following figures we determine acceleration and speed over time and the direction autocorrelation function which help to detect and quantify regularities within trajectories It detects wave-like periodicities, and provides an indication of their wavelength and amplitude. The function C(Δs) is applied to a rediscretized trajectory, and calculates the differences in step angles at all steps separated by ΔΔ, for a range of Δs. The position of the first local minimum in C(Δs) (i.e. the 2-dimensional position (Δs,c(Δs)) may be used to characterise the periodicity within a trajectory.

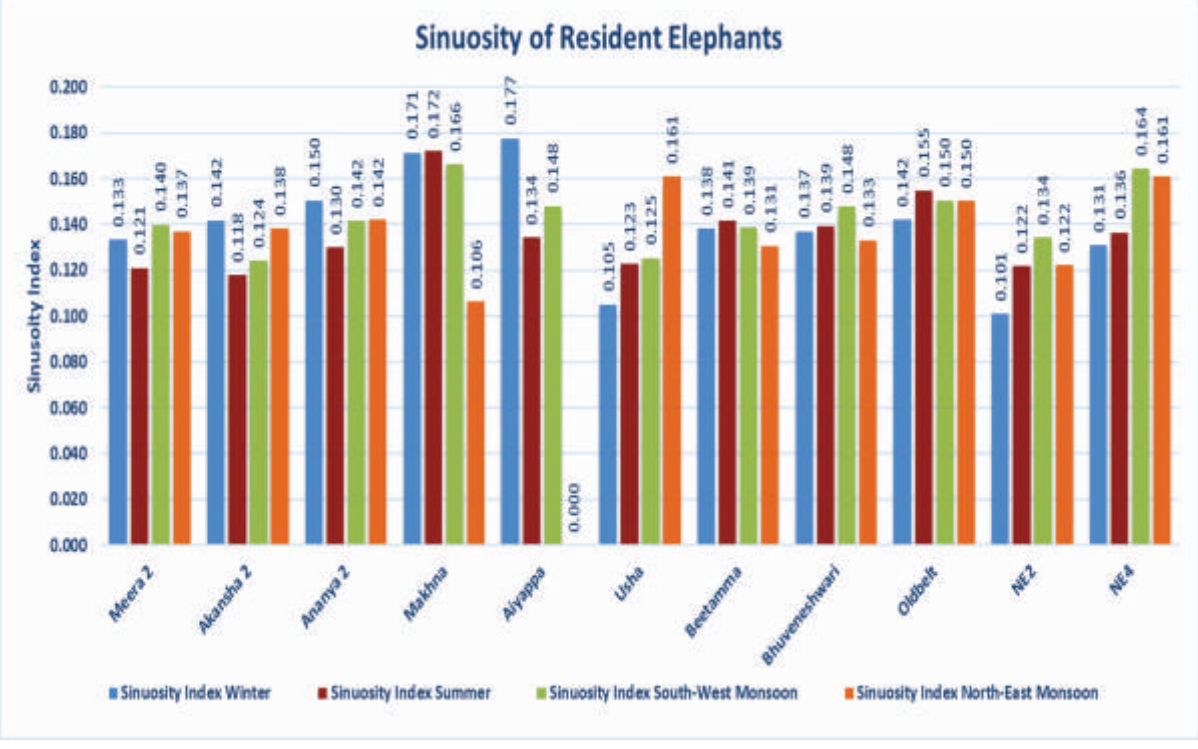
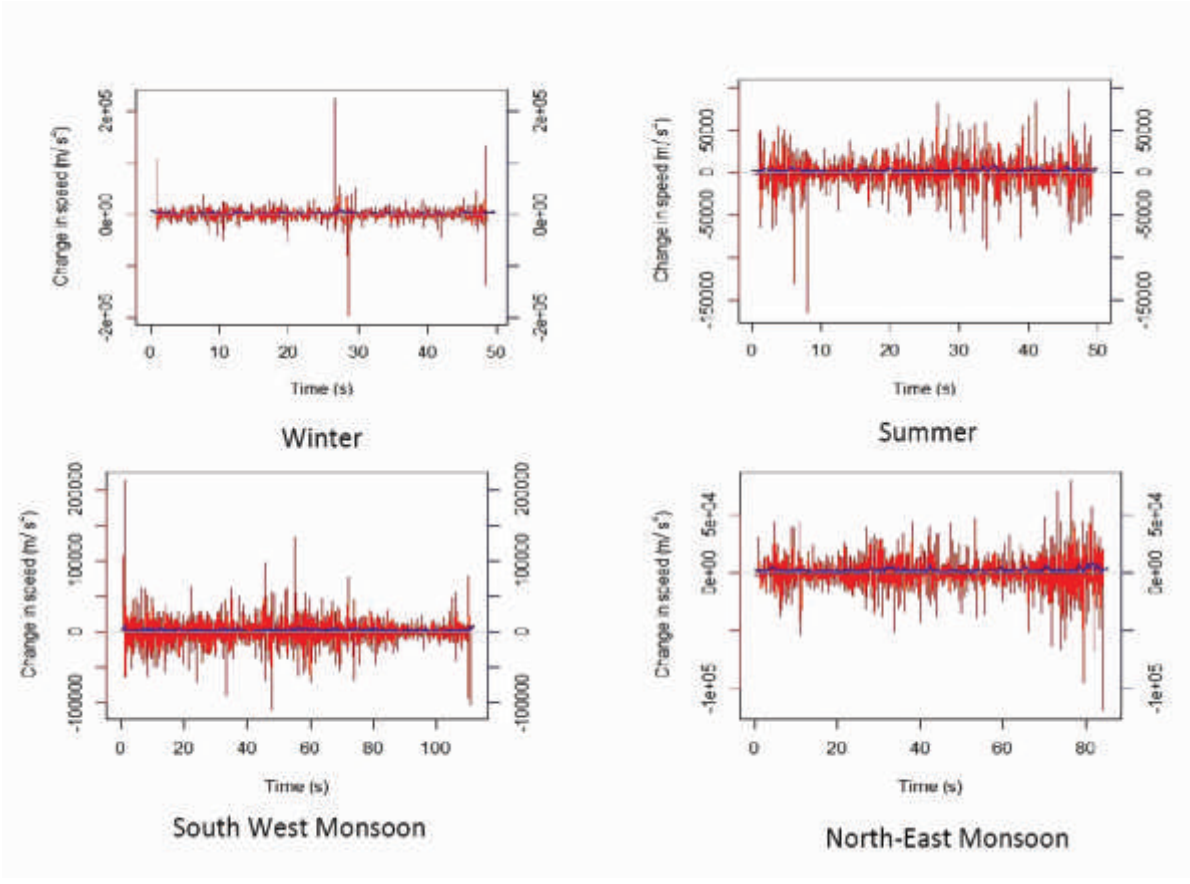


Figure 6.13 : *Seasonal sinuosity index of resident radio-collared individuals*



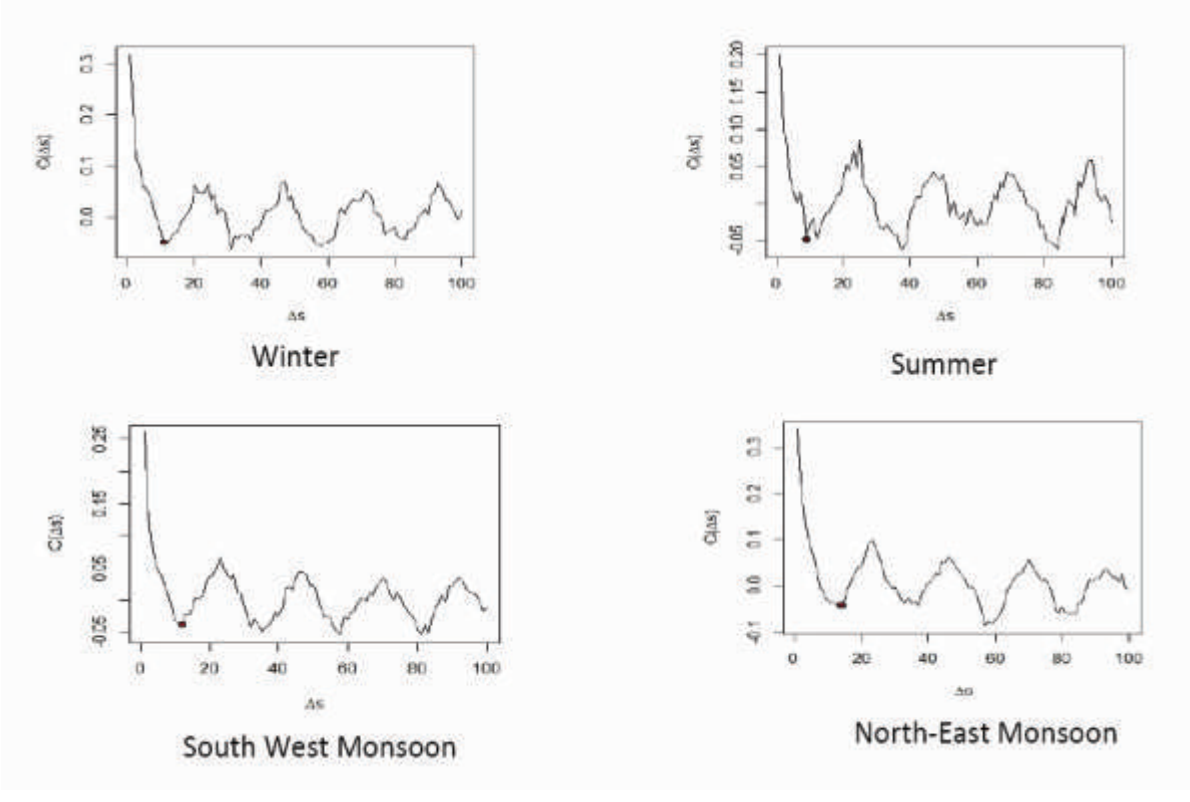


Figure 6.14 : *Acceleration, speed and Direction autocorrelation of trajectory of Ananya 2(female) with respect to season*

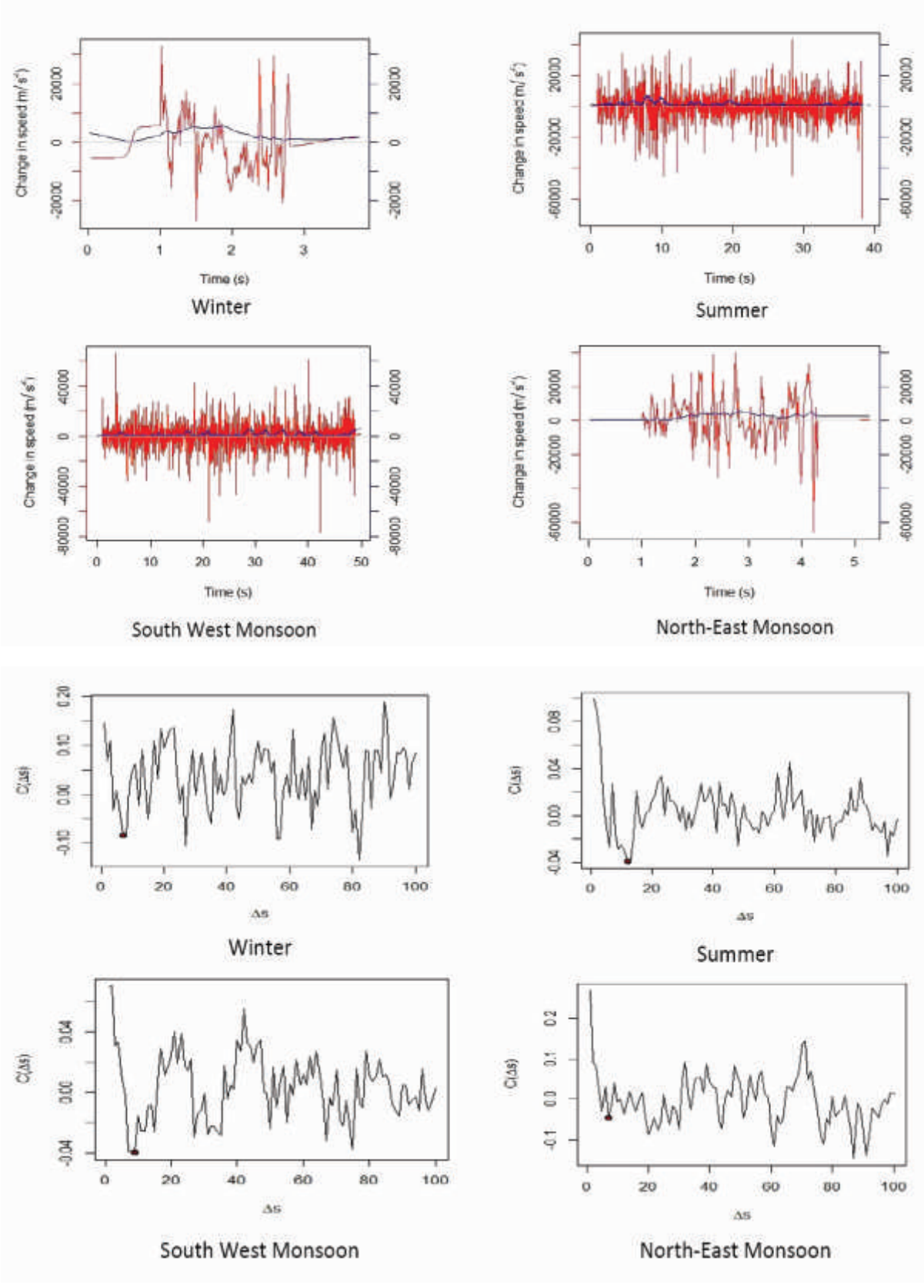


Figure 6.15 : *Acceleration, speed and Direction autocorrelation of trajectory of Makhna (male) with respect to season*

Daily Mean Distance travelled per day

Herds of Kodagu

As the data is parametric we performed the one-way ANOVA test for the three herds of Kodagu namely Akansha 2, Meera 2, Ananya 2, Usha, and two resident males residing in the same area Aiyappa and Makhna along with NE2, which is a Nagarhole Tiger Reserve resident herd (df=6, sum sq= 655, mean sq =109.1, F=34.06, p=0.001) which shows that there is a significant difference in mean distance travelled per day by the radio-collared elephants. To check the pairwise difference between each collared elephant we performed the Tukey HSD test.

Tukey HSD test shows that there is a significant difference between each elephant in terms of distance travelled per day by each elephant and based on the difference of mean distance travelled per day on the adjusted alpha value(p-value)<0.05, there is a significant difference between Akansha 2 and Meera 2(p=0.001), NE2 and Akansha 2 (p=0.001), Ananya 2 and Meera 2 (p=0.001), NE2 and Ananya 2 (p=0.001), Usha and Akansha 2 (p=0.001), Usha and Ananya 2 (p=0.001), Meera 2 and Usha (p=0.02) and Usha and NE2(p=0.01).The male resident Aiyappa also has a difference with Akansha 2 (p=0.003, and Usha (0.001). However, there is no significant difference in distance travelled per day with NE2 (p=0.41). Similarly, Makhna, another resident male also has a significant difference with Ananya(p=0.04), Meera 2 (p=0.001), Usha(p=0.001) and NE2(p=0.001). Makhna and Aiyappa have a significant difference among them (p=0.003).

We also performed pairwise comparison using paired t-tests among these seven individuals and we inferred that the p-value of that of NE2 and Akansha 2 (p=0.001), NE2 and Ananya 2 (p=0.001), NE2 and Usha (p=0.001), Meera and Usha(p=0.002), Ananya 2 and Usha(p=0.001), Akansha 2 and Usha(p=0.001), Ananya 2 and Meera 2(p=0.001), Usha and Makhna(p=0.001), Usha and Aiyappa (p= 0.001), Meera 2 and Makhna(p=0.001), Meera 2 and Akansha(p=0.001), Meera 2 and Aiyappa(p=0.012), Ananya 2 and Akansha 2(p=0.049), Ananya 2 and Makhna(p=0.004), Aiyappa and Ananya 2(p=0.026), Akansha 2 and Aiyappa(p=0.001) and Makhna and Aiyappa(p=0.001) showing the significant difference between the movement patterns in the daily distance travelled per day.

Table 6.12 : Kodagu individual wise summary of movement per day

Individual	Count	Mean (km)	Standard Deviation (km)
Akansha 2	641	3.43	1.56
Ananya 2	642	3.631	1.8
Meera 2	694	4.33	1.75
Usha	231	4.77	2.19
Aiyappa	209	3.96	1.40
Makhna	237	3.22	1.40
NE2	418	4.26	2.24

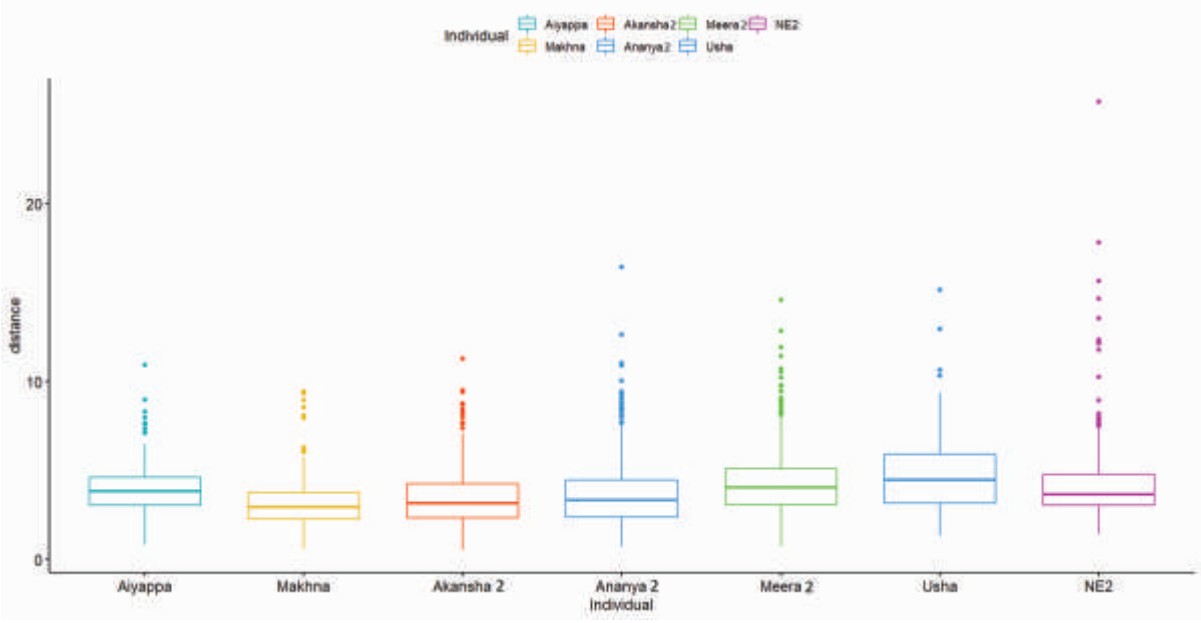


Figure 6.16 : *Daily diurnal displacement of Kodagu individuals with NE2*

Herds of Hassan

Similarly in Hassan, three herds namely old belt, Beetamma, and Bhuveneshwari were also compared with NE2 by performing a one-way ANOVA test (df=3, sum sq= 121, mean sq =40.32, F=11.4, p= 0.001) thereby signifying the significant difference among them in terms of mean distance travelled per day.

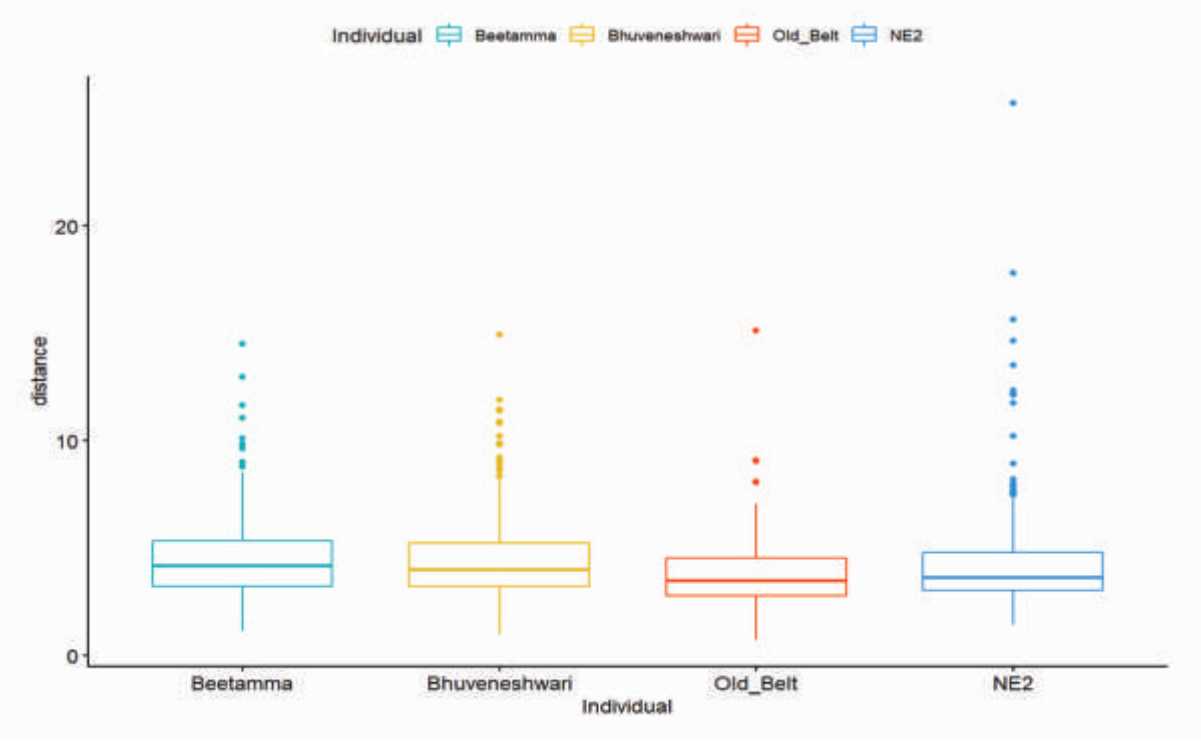


Figure 6.17 : *Data visualisation for daily diurnal displacement of Hassan individuals with NE2*

Table 6.13 : Hassan individual wise summary of movement per day

Individual	Count	Mean (km)	Standard Deviation (km)
Beetamma	443	4.459	1.76
Bhuveseshwari	437	4.48	1.89
Old_Belt	303	3.738	1.46
NE2	418	4.26	2.24

Tukey HSD test shows were also performed to check pairwise difference to see if there is a significant difference between each elephant in terms of distance travelled per day by each elephant and based on the difference of mean distance travelled per day on the adjusted alpha value(p-value)<0.05, there is a significant difference between Oldbelt and Beetamma (p=0.001), Oldbelt and Bhuveneshwari (p=0.001), NE2 and Oldbelt (p=0.001) in the distance travelled per day.

Paired t-test showed us that there is a significant difference in mean distance travelled between NE2 and Oldbelt (p=0.001), Beetamma and Oldbelt (p=0.001), and Oldbelt and BHuveneshwari (p=0.001) whereas there is no such significant difference between NE2 and Beetamma (p=0.15) and NE2 and Bhuveneshwari (p=0.139).

Herds of Protected area (Nagarhole Tiger Reserve)

Two herds namely NE2 and NE4 were radio-collared within the protected area of Nagarhole Tiger Reserve. From the One-way Anova test (df=1, sum sq= 46.3, mean sq =46.27, F=13.63, p= 0.001) thereby signifying the significant difference among them in terms of mean distance travelled per day.

Table 6.14 : NTR individual wise summary of movement per day

Individual	Count	Mean (km)	Standard Deviation (km)
NE2	418	4.26	2.24
NE4	414	3.79	1.33

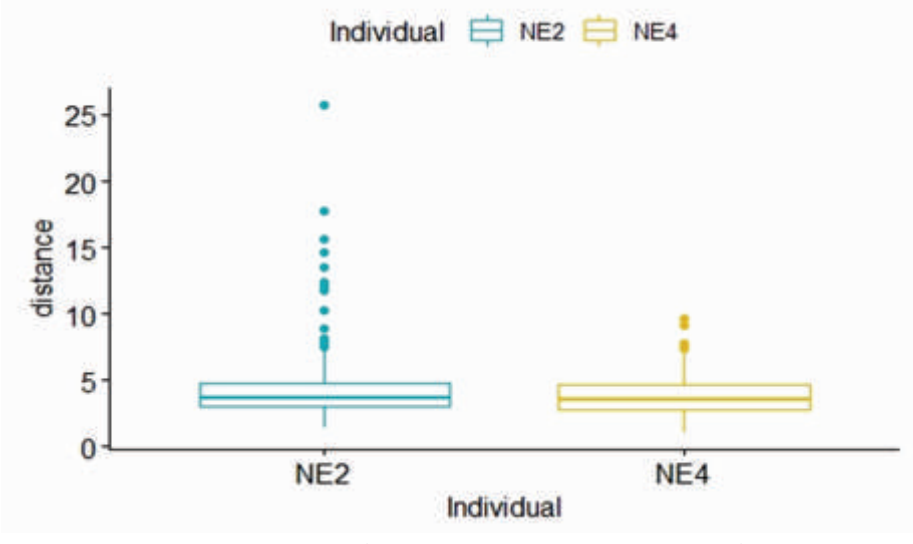


Figure 6.18 : *Data visualisation for daily diurnal displacement of NTR Individuals*

Tukey HSD test were also performed to check pairwise difference to see if there is a significant difference between each elephant in terms of distance travelled per day by each elephant and based on the difference of mean distance travelled per day on the adjusted alpha value(p-value)<0.05, there is a significant difference between NE2 and NE4 (p=0.001) in the distance travelled per day. Similarly, pairwise comparison by paired t-test showed that there is a significant difference in distance travelled per day (p=0.001).

Distance travelled per day and night

We performed statistical analysis on the distance travelled by elephants per day and night to understand if there is a significant difference between them considering two variables one being the day and night difference in mean and the other being the difference in individuals. So, we performed a two-way ANOVA test to check the significant difference in the mean travelled by elephants. The analysis was performed in R statistical software (4.2).

Herds of Kodagu vs NE2

Here the herds of Kodagu are compared with NE2 which was collared in the protected boundary of Nagarhole Tiger Reserve. The result obtained for variable time (day and night) is df=1, sum sq=70, mean sq=69.71, F= 57.71, p= 0.001 and Individual(elephants) is df= 6, sum sq=323, mean sq= 53.89, F= 44.61, p= 0.001, which thereby signifies that there is a significant difference between the mean distance travelled by elephants during day and night time, where individual being the more significant variable.

Multiple pairwise comparisons were carried out with Tukey HSD and paired t-tests were conducted for post hoc comparison between the groups. Tukey HSD test shows that there is a significant difference between each elephant in terms of distance travelled per day and night by each elephant and based on the difference of mean of distance on the adjusted alpha value(p-value)<0.05, there is a significant difference between Meera 2 and Akansha 2 (p=0.001), NE2 and Akansha 2 (p=0.001),Meera 2 and Ananya 2 (p=0.001),NE2 and Ananya 2 (p=0.001), Akansha 2 and Aiyappa(p=0.001), Makhna and Aiyappa (p=0.001), Usha and Aiyappa(p=0.001), Usha and Akansha 2 (p=0.001), Makhna and Ananya 2 (p=0.011), Usha and Ananya 2 (p=0.001), Meera 2 and Makhna (p=0.001), NE2 and Makhna 2(p=0.001), Usha and Makhna(p=0.001), Usha and Meera 2 (p=0.004) , Usha and NE2(p=0.002) in the distance traveled per day and night by radio-collared female elephants.

Table 6.15 : Kodagu individual wise summary of day and night movement

Individual	Day			Night		
	Count	Mean(km)	SD (km)	Count	Mean(km)	SD (km)
Akansha 2	638	1.61	0.924	641	1.82	0.967
Ananya 2	641	1.644	0.95	642	1.989	1.301
Meera 2	694	2.059	1.098	694	2.268	1.089
Usha	231	1.95	1.12	231	1.95	1.12
Aiyappa	209	1.68	0.795	209	2.29	0.994
Makhna	236	1.52	0.716	237	1.71	0.986
NE2	418	2.39	1.37	418	1.87	1.19

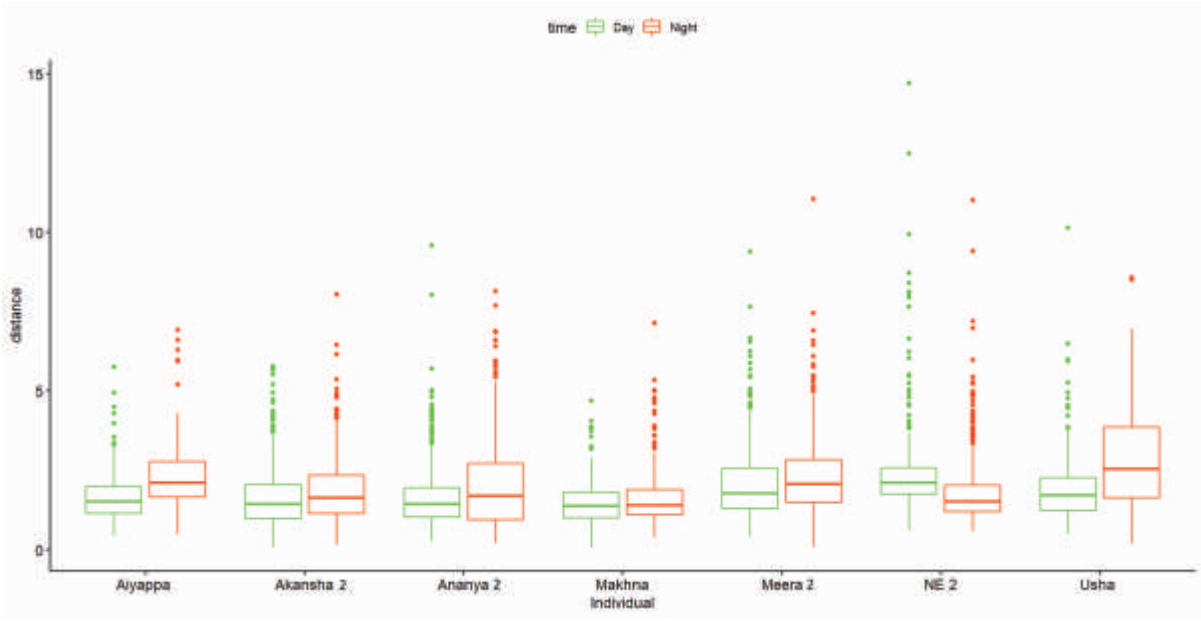


Figure 6.19 : *Data visualisation for daily diurnal and nocturnal displacement of Kodagu Individuals with NE2*

Whereas, paired t test showed that that there is a significant statistical difference between NE2 and Akansha 2 and Aiyappa(p=0.001), Aiyappa and Ananya 2 (p=0.001), Ananya 2 and Akansha 2 (p=0.029), Makhna and Aiyappa(p=0.001), Makhna and Ananya 2 (p=0.001), Meera 2 and Aiyappa (p= 0.005), Meera 2 and Akansha 2 (p= 0.001), Meera 2 and Ananya 2 (p=0.001), Makhna and Meera 2 (p=0.001), NE2 and Aiyappa(p=0.03), NE2 and Akansha 2 (p=0.001), NE2 and Ananya 2 (p=0.001), Makhna and NE2(p=0.001), Usha and Aiyappa(p=0.001), Usha and Akansha 2 (p= 0.001), Usha and Ananya 2 (p= 0.001), Usha and Makhna(p= 0.001), Usha and Meera 2 (p=0.001), Usha and NE2(p= 0.001) as per distance travelled during day and night by these respective individuals.

Hassan herds vs NE2

Similarly, the herds radio-collared in Hassan are compared with NE2 which was radio-collared in the protected boundary of Nagarhole Tiger Reserve. The result obtained for variable time (day and night) is df=1, sum sq=103, mean sq=103.38, F= 70.88, p= 0.001 and Individual(elephants) is df= 3, sum sq=60, mean sq= 20.16, F= 13.82, p= 0.001, which thereby signifies that there is a significant difference between the mean distance travelled by elephants during day and night time, where time being the more significant variable.

Multiple pairwise comparisons were carried out with Tukey HSD and paired t-tests were conducted for post hoc comparison between the groups. Tukey HSD test shows that there is a significant difference between each elephant in terms of distance travelled per day and night by each elephant and based on the difference of the mean of distance on the adjusted alpha value(p-value) <0.05, there is a significant difference between Oldbelt and Beetamma (p=0.001), Oldbelt and Bhuveneshwari (p=0.001),Oldbelt and NE2 (p=0.001) as per the distance travelled per day and night by radio-collared female elephants.

Table 6.16 : Hassan individual wise summary of day and night movement

Individual	Day			Night		
	Count	Mean(km)	SD (km)	Count	Mean(km)	SD (km)
Beetamma	443	1.859	0.939	442	2.602	1.316
Bhuveneshwari	437	1.973	0.907	437	2.506	1.48
Oldbelt	303	1.489	0.913	303	2.249	1.07
NE2	418	2.39	1.37	418	1.87	1.19

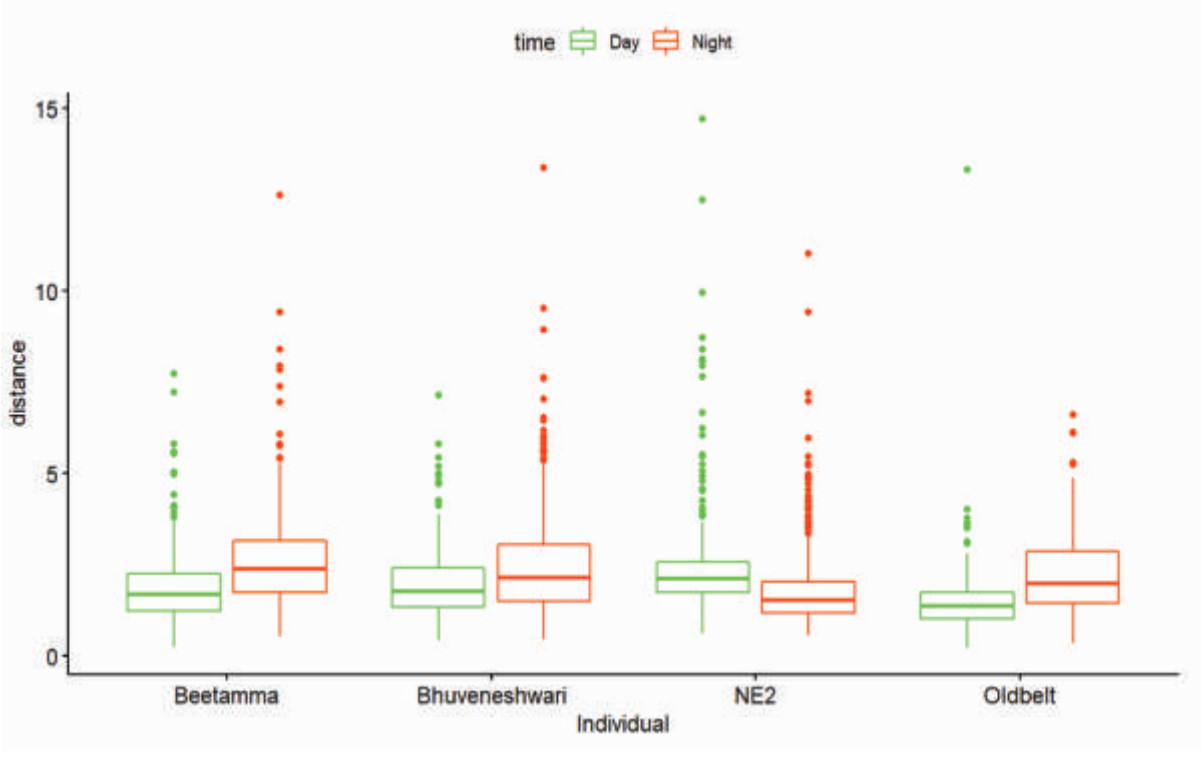


Figure 6.20 : *Daily diurnal and nocturnal displacement of Hassan Individuals with NE2*

Whereas, a paired t-test showed that there is a significant statistical difference between NE2 and Oldbelt (p=0.001), Oldbelt and Beetamma(p=0.001), Oldbelt and Bhuveneshwari (p=0.001) as per distance travelled during day and night by these respective individuals, whereas NE2 and Beetamma (p=0.11) and NE2 and Bhuveneshwari(p=0.1) didn't have a significant difference between the individuals.

NE2 vs NE4

Similarly, the herds radio-collared in Nagarhole Tiger Reserve were compared among themselves The result obtained for variable time (day and night) is df=1, sum sq=7.6, mean sq=7.616, F= 6.022, p= 0.014 and Individual(elephants) is df= 1, sum sq=23.1, mean sq= 23.135, F= 18.291, p=0.001, which thereby signifies that there is a significant difference between the mean distance travelled by elephants during day and night time, where individual being the more significant variable.

Table 6.17 : NTR individual wise summary of day and night movement

Individual	Day			Night		
	Count	Mean (km)	SD (km)	Count	Mean (km)	SD (km)
NE2	418	2.39	1.37	418	1.87	1.19
NE4	414	1.77	0.795	414	2.02	0.989

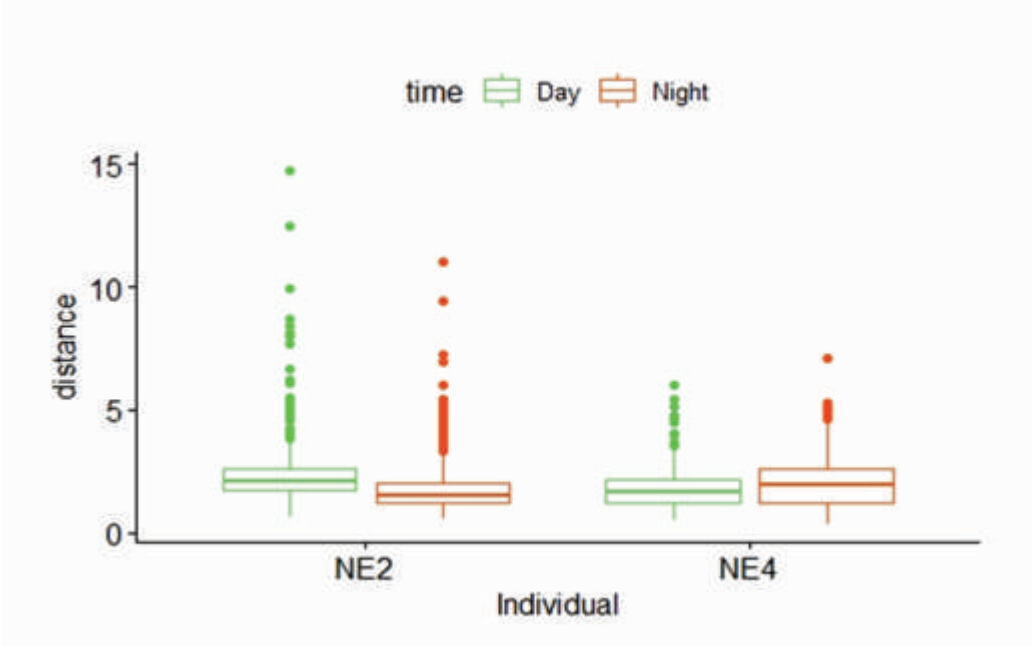


Figure 6.21 : *Data visualisation for daily diurnal and nocturnal displacement of NTR individual*

Tukey HSD test shows that there is a significant difference between each elephant in terms of distance traveled per day and night by each elephant and based on the difference of the mean of distance on the adjusted alpha value(p-value) <0.05, there is a significant difference between NE2 and NE4(p=0.001). Paired t-test also implied the same between these individuals (p=0.001) stating the significant difference between the individuals in terms of distance travelled during day and night.

Habitat composition of radio-collared individual

We also deduced the habitat composition of each radio-collared herd by superimposing the home range (100% MCP) radio-collared individuals on LULC of Karnataka and deduced the area in Arc GIS 10.5, which forest type they spend the majority of their time from their home range. From the graph below we can see that all radio-collared individuals spend the majority of their time in the plantation area except for NE2 (Deciduous Forest), which was radio-collared in Nagarhole Tiger Reserve, though sometimes it strays to the plantation for easy food. NE4 spends majorly in plantation areas mostly and in a forest area. The radio-collared individuals of Hassan mostly accessed fragmented patches spend majorly in plantation areas and time to time raided croplands as well. The Kodagu herds namely Akansha 2, Meera 2 and male resident herd Aiyappa spend the majority of their time on the plantation whereas Ananya 2, Usha and Makhna accesses both plantation areas and forest areas as well.

Figure 6.22 : *Habitat composition of resident radio-collared individuals*

Habitat Availability and Utilisation

Habitat selection by wildlife is an important aspect of ecology. Studies of habitat selection provide information on environmental characteristics needed by animals, essential knowledge for the development of wildlife management, and conservation policies. The 100% MCP is our available area and the used area is calculated from 95% LSCV as it deems to be a better-fitted model. The available and used area is extracted from LULC of Karnataka using Arc GIS 10.5. The analysis was done in R statistical software 4.2 using the adehabitatHS package. Significance of selectivity ratio (wi) was determined using loglikelihood and 95% confidence intervals for each habitat category; a selectivity ratio >1 indicates disproportionate preference, and values

Kodagu radio-collared individuals

Manly's selectivity measures (wi) (selection ratio: used/available) were calculated for elephant individuals in Kodagu. Global selection ratios showed no significant difference in the habitat selection for the elephants (log-likelihood=9.648; df=23, p=0.99). Deciduous forest and plantation were found to be used more than their availability. Akansha 2, Ananya 2 and Usha use deciduous forests more than its available whereas Makhna (resident male) and Meera 2 uses and plantation area more than its available. Usha and Ananya 2 uses plantation area less than its available. Akansha 2 was also observed to use cropland more than is available. The herds mostly stray away from the natural habitat into plantations and cropland. This behaviour is likely driven by the availability of food and refuge in these areas. The elephants may be attracted to these habitats due to the easy accessibility of resources.

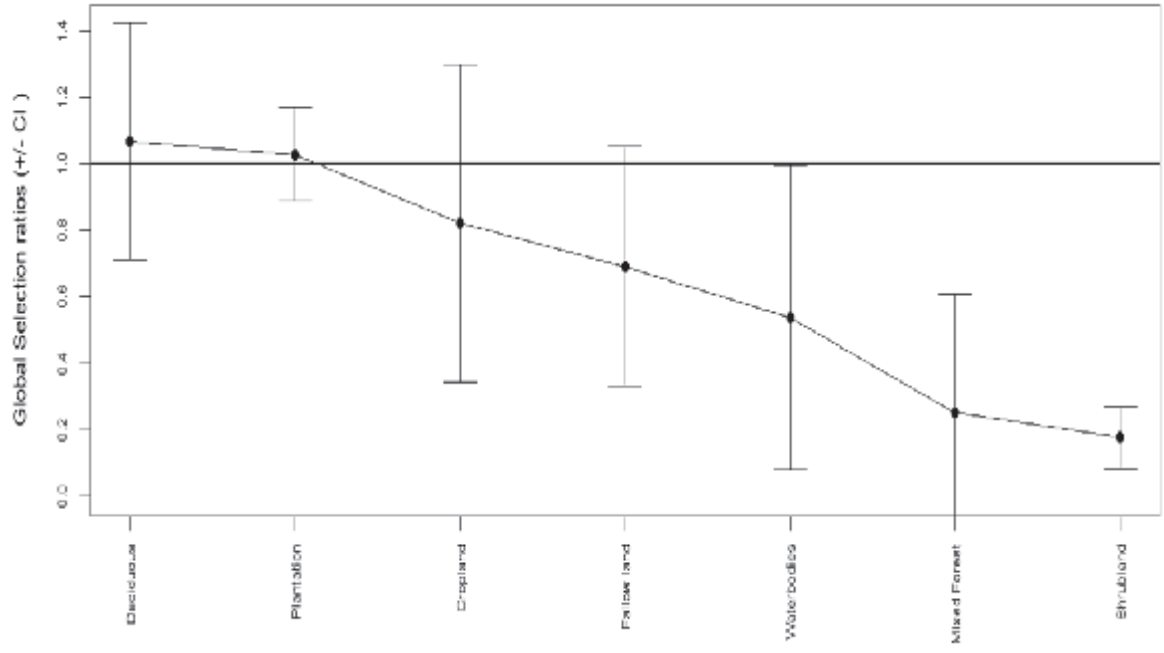


Figure 6.23 : *Global selection ratios of Kodagu individuals*

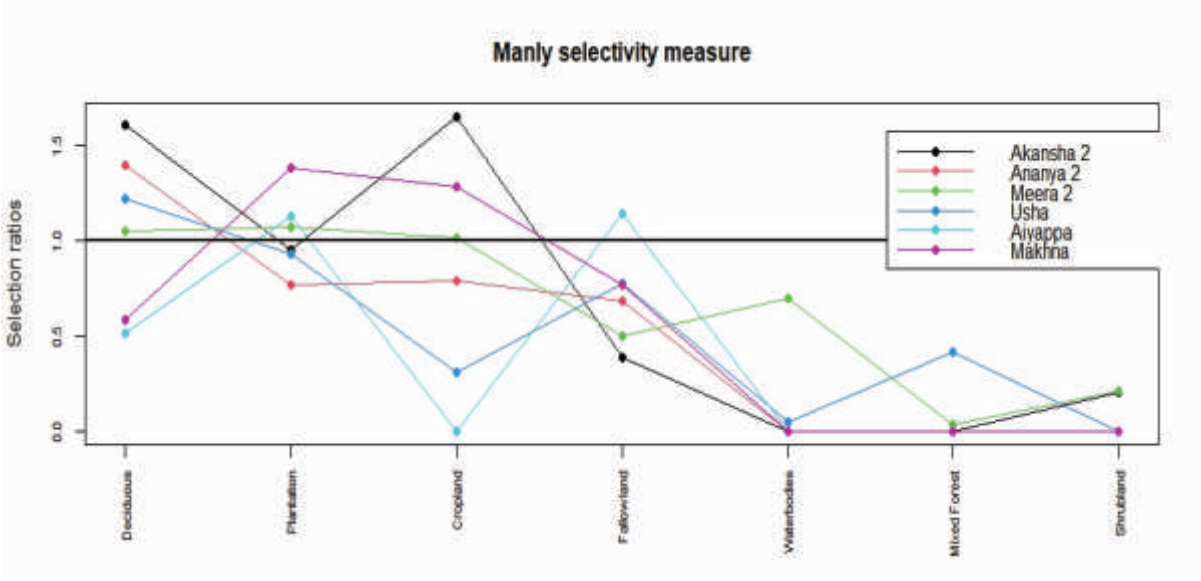


Figure 6.24 : *Individual selection ratios of Kodagu Individuals*

Hassan radio-collared individuals

The habitat selection of elephant individuals in Hassan was analysed using Manly's selectivity measure (wi), which calculates the selection ratio of used habitat compared to available habitat. The global selection ratios showed no significant difference in habitat selection for the elephants (log-likelihood = 9.01, df-17, p=0.93). Plantations and croplands were utilized more frequently by the elephants compared to their availability. This preference can be attributed to their home range being located within fragmented patches where plantations provide a readily available source of food and refuge. These areas likely offer an abundance of resources, making them attractive to the elephants. However, the reliance on plantations can contribute to increased instances of human-elephant conflict, as these areas are often in close proximity to human settlements.

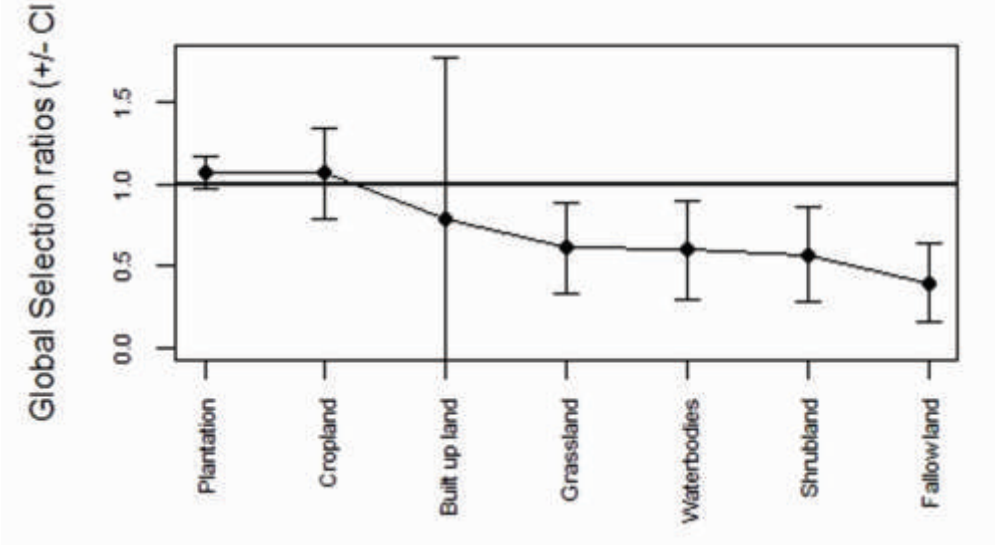


Figure 6.25 : *Global selection ratios of Hassan Individuals*

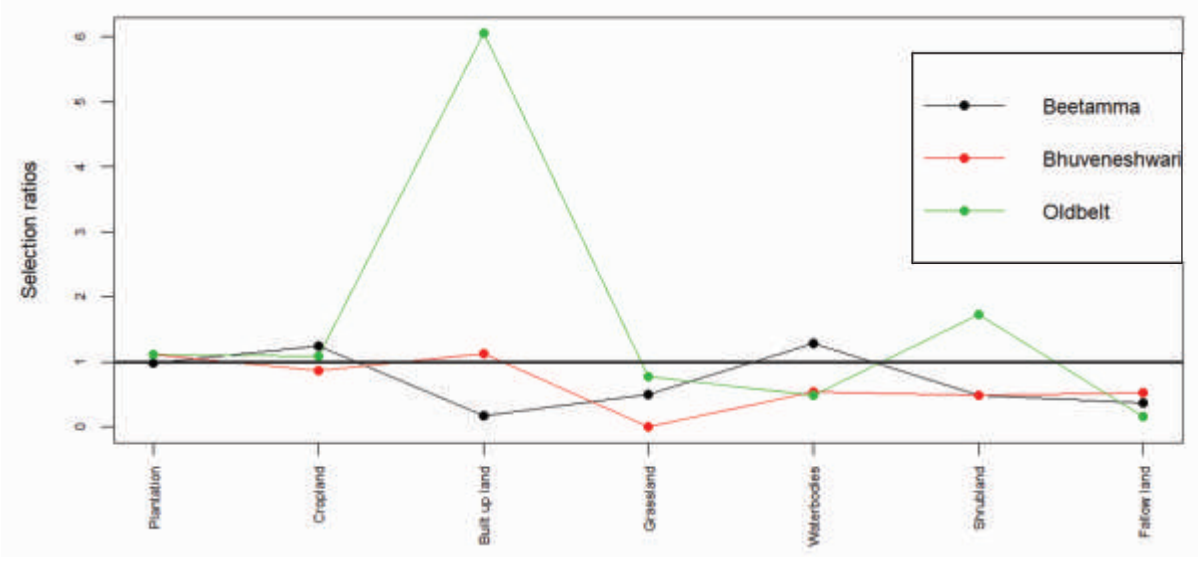


Figure 6.26 : *Individual selection ratios of Hassan Individuals*

Radio-collared individuals of Nagarhole

Manly's selectivity measures (wi) (selection ratio: used/available) were calculated for elephant individuals in Nagarhole Tiger Reserve. Global selection ratios showed no significant difference in the habitat selection for the elephants (log-likelihood=7.62, df-7, p=0.366). Deciduous forest was found to be used more than its availability. Occasional movement of these elephants into plantations can be attributed to the proximity of plantations to forested areas, offering them easy access to food resources. Elephants are known to adapt their foraging behaviours based on the availability of food and refuge. By recognizing their utilization of adjacent plantations and considering the factors that attract them to these areas, strategies can be developed to mitigate conflicts and promote coexistence between elephants and local communities.

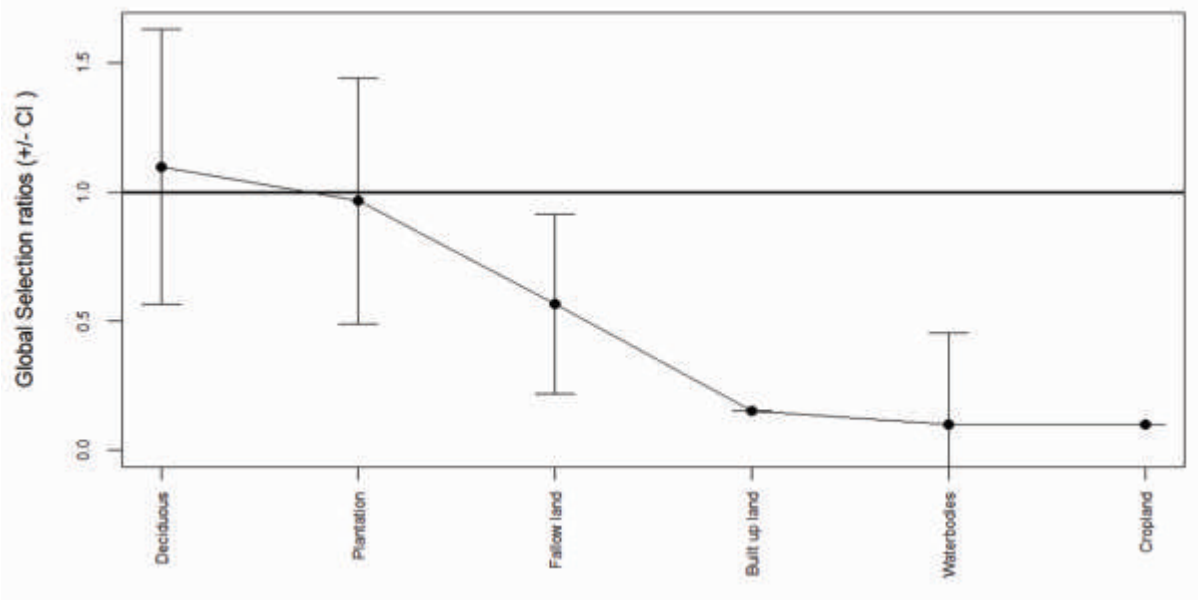


Table 6.27 : *Global selection ratios of NTR radio-collared individuals*

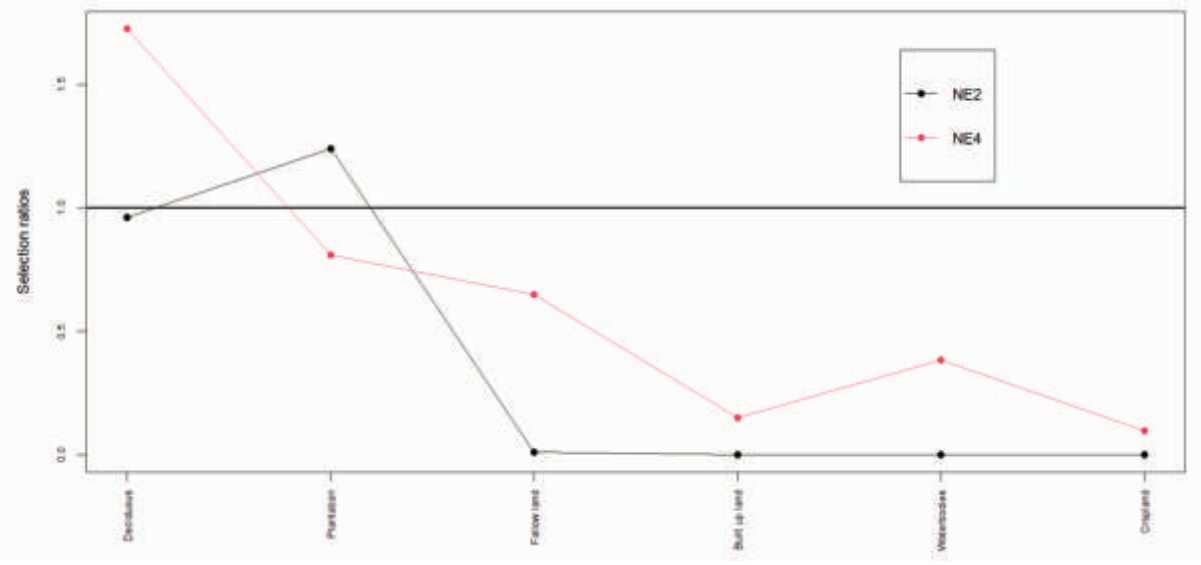


Figure 6.28 : *Individual selection ratios of NTR Individuals*

6.3 Ranging pattern and habitat use of translocated radio-collared elephants (2020-2022)

Home range estimation

All the collared elephants were found inside the protected areas (PAs) along with the adjacent and agricultural patches, except for the Mountain, which was found in Couveri and Medikeri WLS. NE1 and NE3 were observed in Rajiv Gandhi (Nagarhole) NP, Haveri in Bhadra WLS, Chota Bheem in BRT and Cauvery WLS, Basava and Old Makhna in Bandipur and Nagarhole National Park. The 100% MCP for Gunda and Colonel were extended in some parts of Tamil Nadu, Kusha's 100% MCP was extended in Kerala in Wayanad WLS and CKM in Tamil Nadu Mudumalai NP. All the translocated elephants, except NE1 and NE3 (resident elephants), were observed to be still exploring the new area, as the home ranges of elephants have not stabilized yet, since there is an increase in the areas and distance moved in fortnightly and monthly home ranges (MCP; 95% and 50%) and mean daily displacement in a month (Figure 6.29). The no. of locations was less than three months for Mountain, Old Makhna and Basava; hence, they have been excluded from the home range stabilization analysis.

Home range estimates of the collared elephants varied greatly between different estimators (MCP and KDE bandwidths), contours, and BBMM. Mean home range estimates of 100% MCP exceeded the KDE (LSCV) estimates and BBMM. Some elephants' home ranges were found to overlap, but the core ranges, i.e., 50% BBMM and 50% LSCV were exclusive for each elephant. The average 100% MCP home range, was highest (4034.98 sq. km) for Old Makhna (translocated male). In contrast, it was lowest at 454.51 sq. km for Colonel (translocated male) (Table 39). However, the Average 100% MCP for the resident male elephants was significantly less than the translocated males (224.06 and 249.44 sq. km for NE1 and NE3, respectively). A detailed description of the results is presented in Table 6.18, Figure 6.30-6.41.

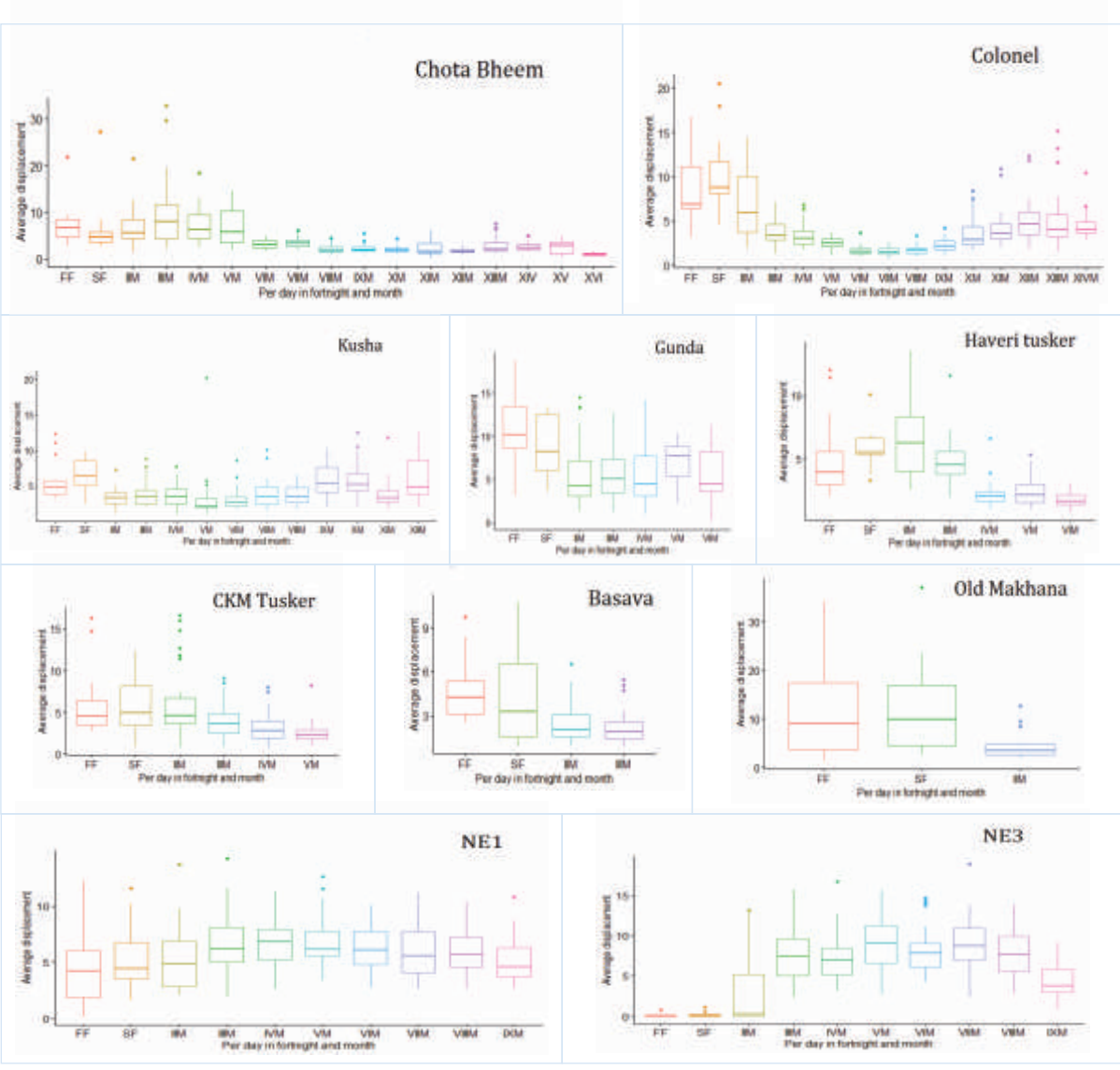


Figure 6.29 : *Mean daily displacement per first two fortnightly and consequent months by translocated and resident elephants in Karnataka*
(FF: First fortnightly; SF: Second fortnightly; IIM: Second Month; IIIM: Third Month, and so on).

Table 6.18 : Home range (95%) and Core range (50%) of translocated elephants in Karnataka.

Elephant ID	No. of days	No. of fixes	MCP 100% (sq. km)	MCP 95% (sq. km)	MCP 50% (sq. km)	KDE LSCV 95% (sq. km)	KDE LSCV 50% (sq. km)	BBMM 95% (sq. km)	BBMM 50% (sq. km)
Chota Bheem	474	10590	1655.25	944.08	111.92	114.12	4.74	193.92	6.30
Colonel	425	8972	454.51	266.86	7.84	35.61	2.51	42.64	2.81
Gunda	180	4788	786.35	594.91	246.63	140.02	10.17	96.00	5.63
Haveri Tusker	178	4084	768.19	566.97	198.32	119.81	5.47	61.98	2.44
Kusha	363	7596	1283.72	1187.72	643.17	331.37	50.20	162.49	17.74
CKM Tusker	150	3388	404.16	334.01	167.49	88.03	11.26	65.32	6.14
Mountain	16	623	721.02	357.41	59.03	91.10	11.19	64.34	4.15
Old Makhna	66	1514	4034.98	3937.68	1022.16	666.46	72.89	156.73	7.96
Basava	92	2094	850.84	832.84	182.04	222.94	24.72	39.79	3.21
NE1 (Resident)	287	7041	224.06	118.46	13.72	37.90	5.05	43.53	5.20
NE3 (Resident)	285	8393	249.44	183.27	43.84	64.50	6.72	74.76	8.80

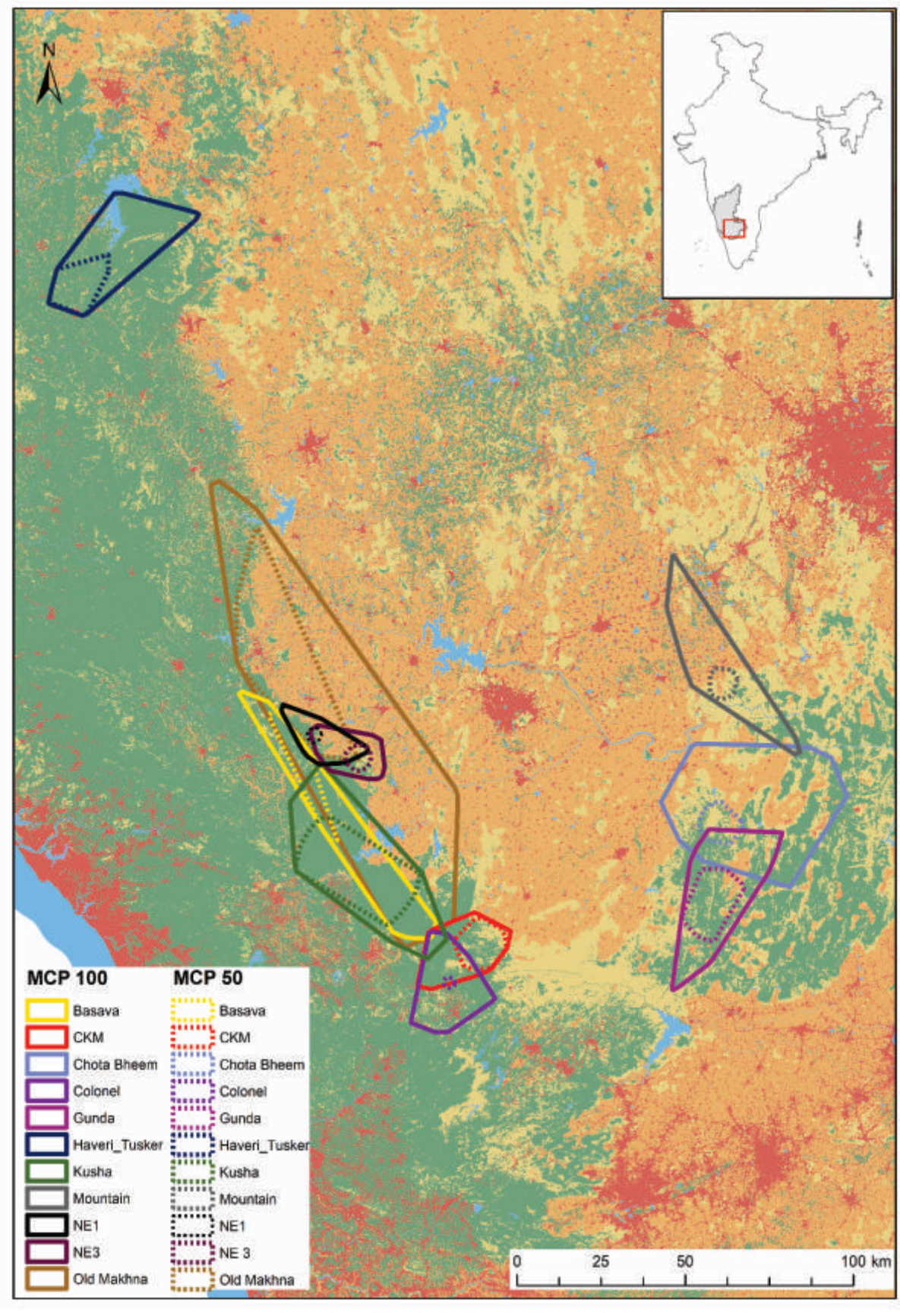


Figure 6.30 : *MCP (100% and 50%) for the translocated and resident male elephant in Karnataka.*

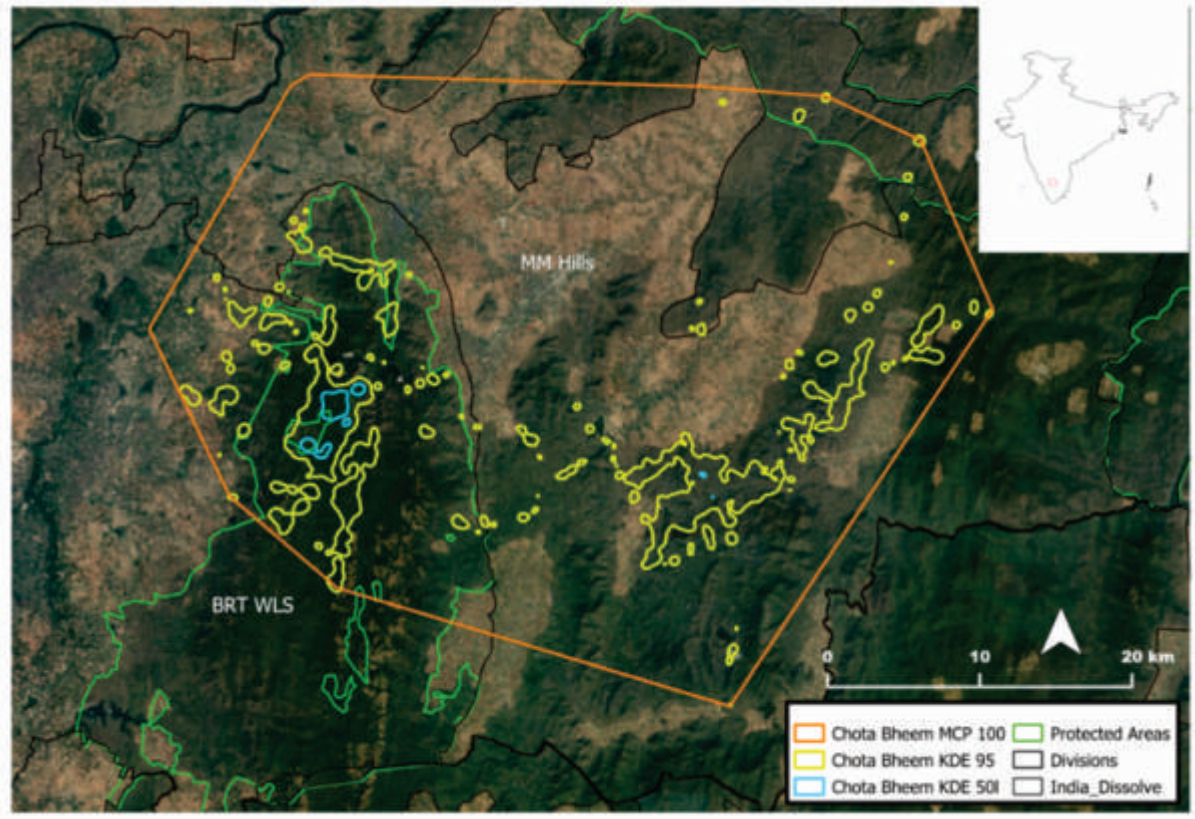


Figure 6.31 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Chota Bheem.*

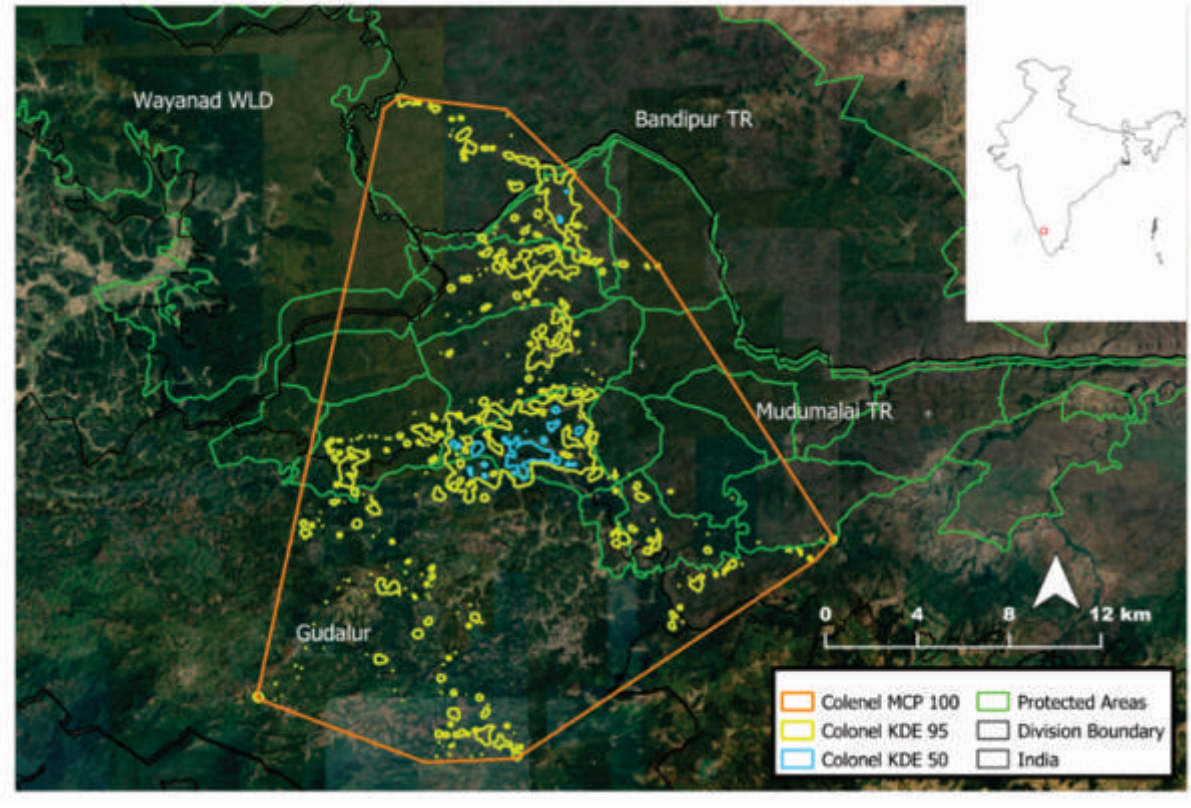


Figure 6.32 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Colonel.*

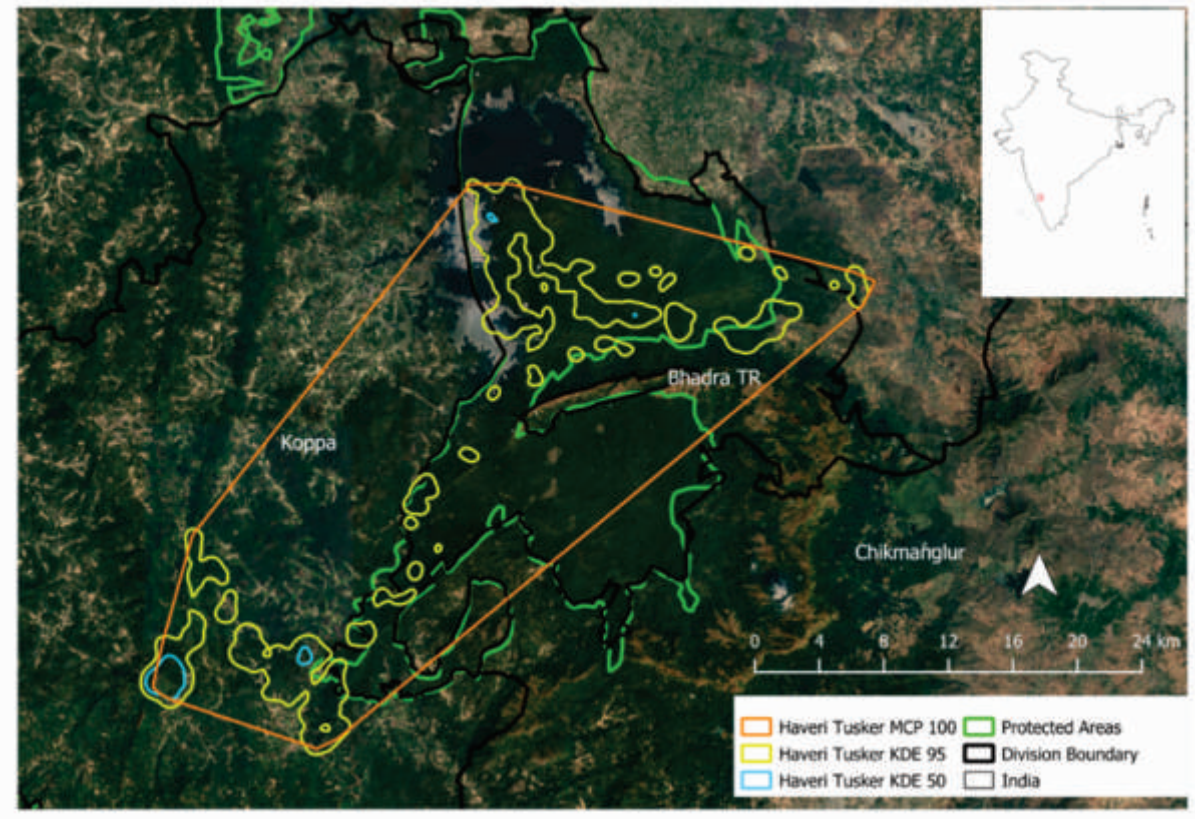


Figure 6.33 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Haveri Tusker.*

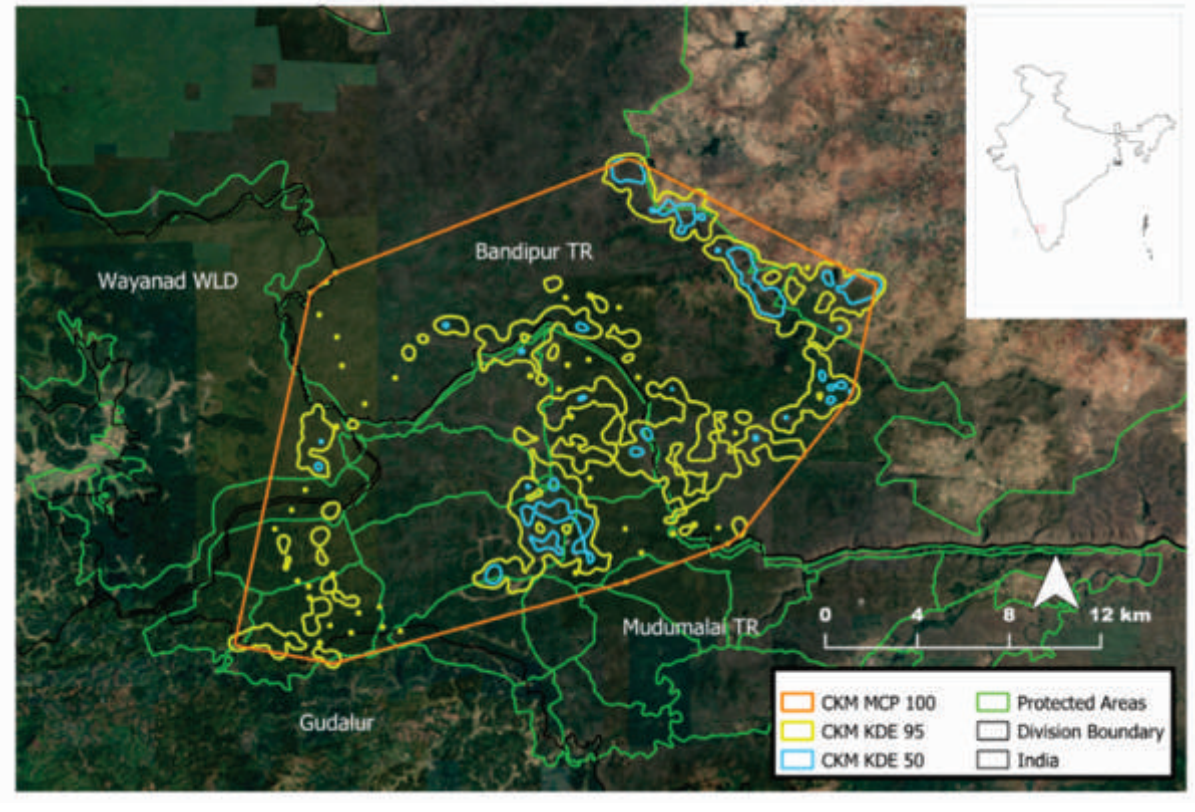


Figure 6.34 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant CKM Tusker.*

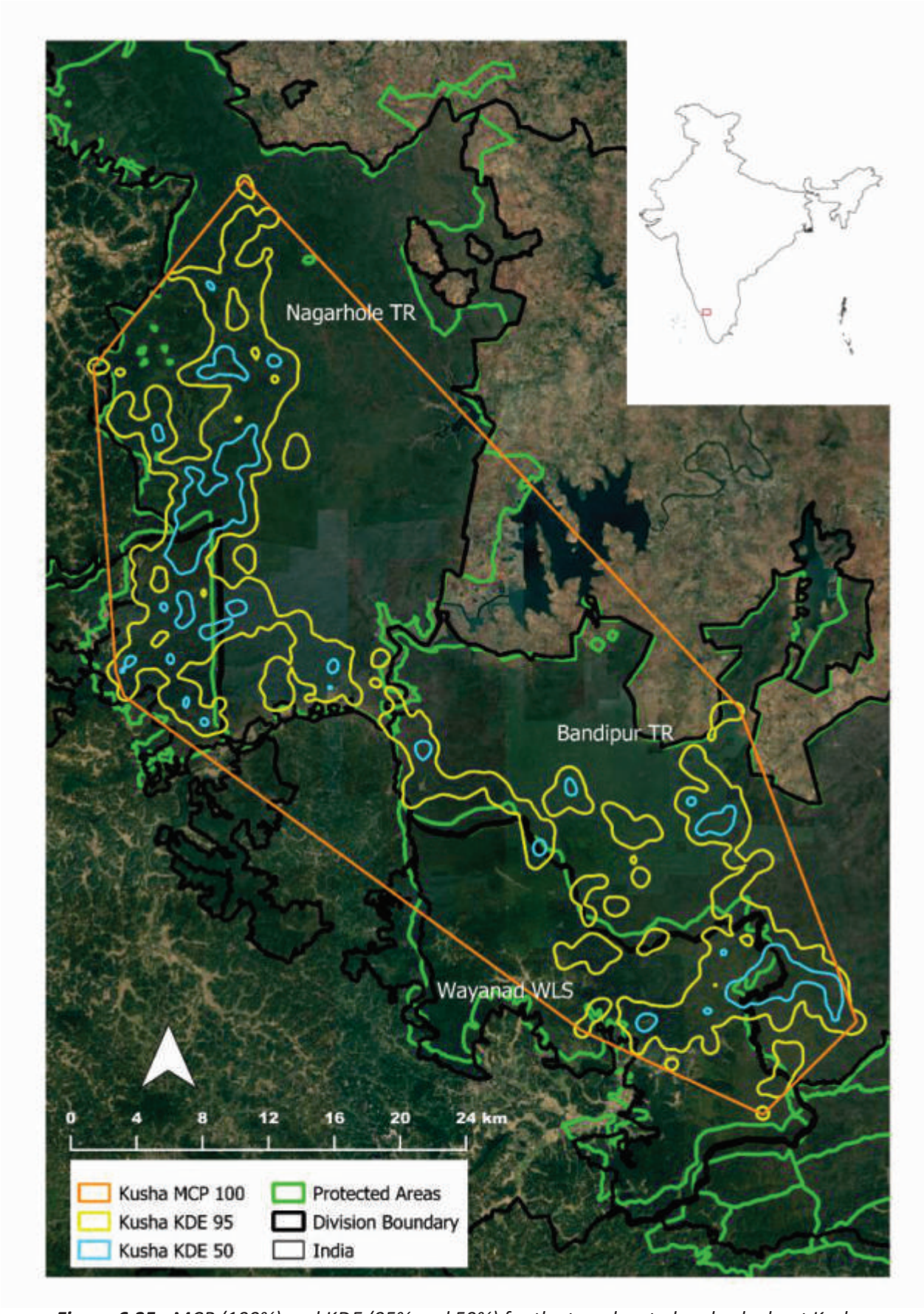


Figure 6.35 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Kusha.*

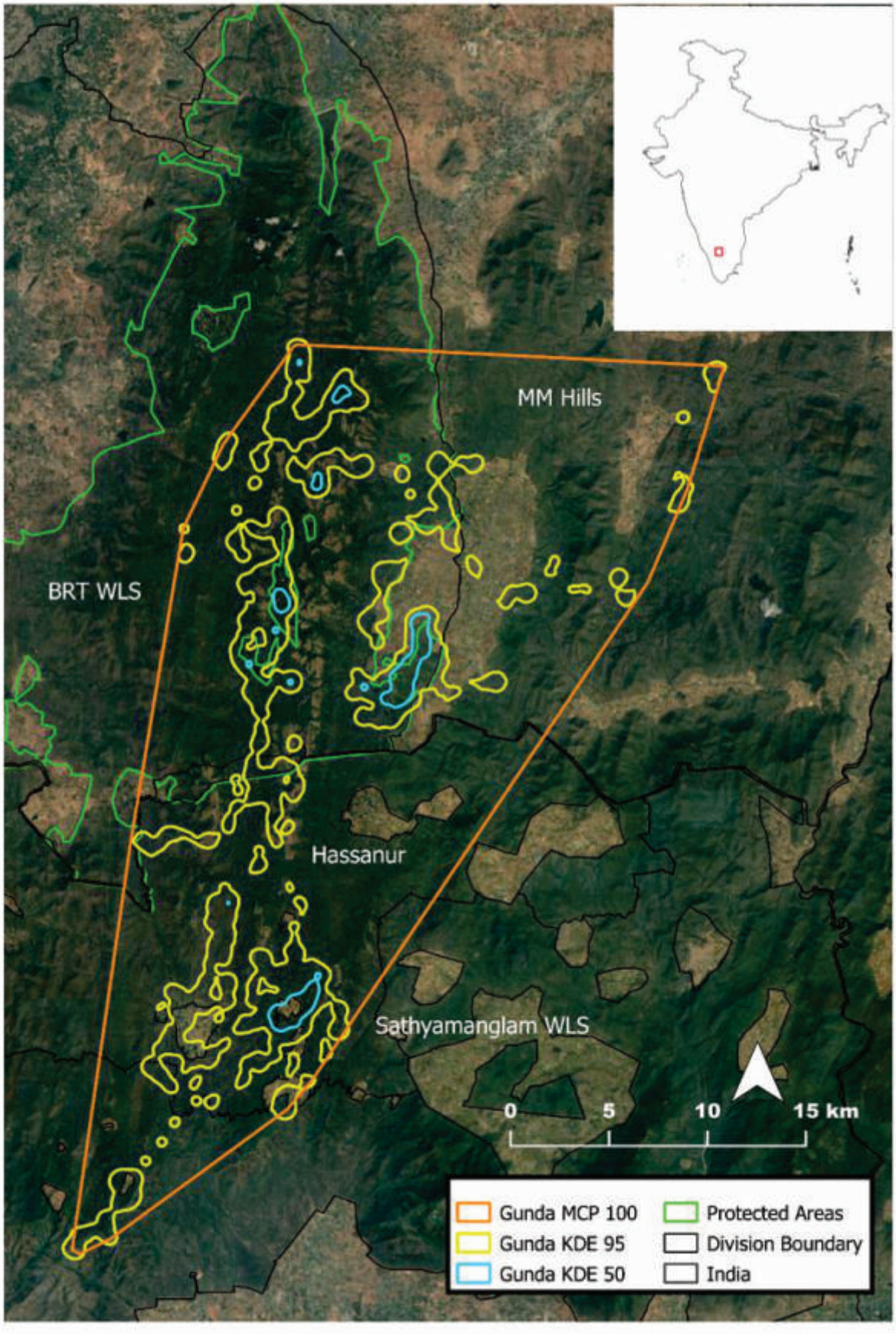


Figure 6.36 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Gunda.*

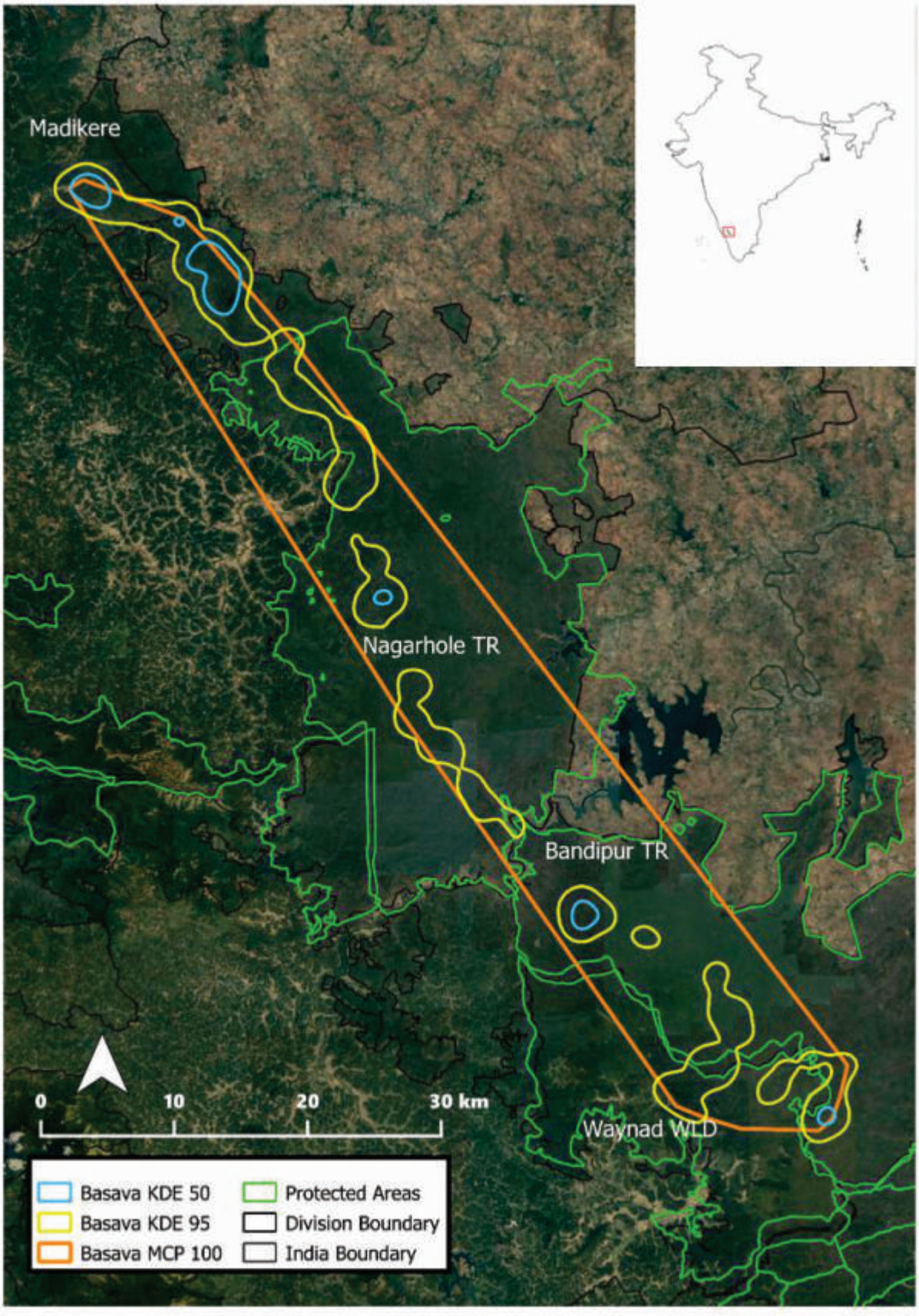


Figure 6.37 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Basava.*

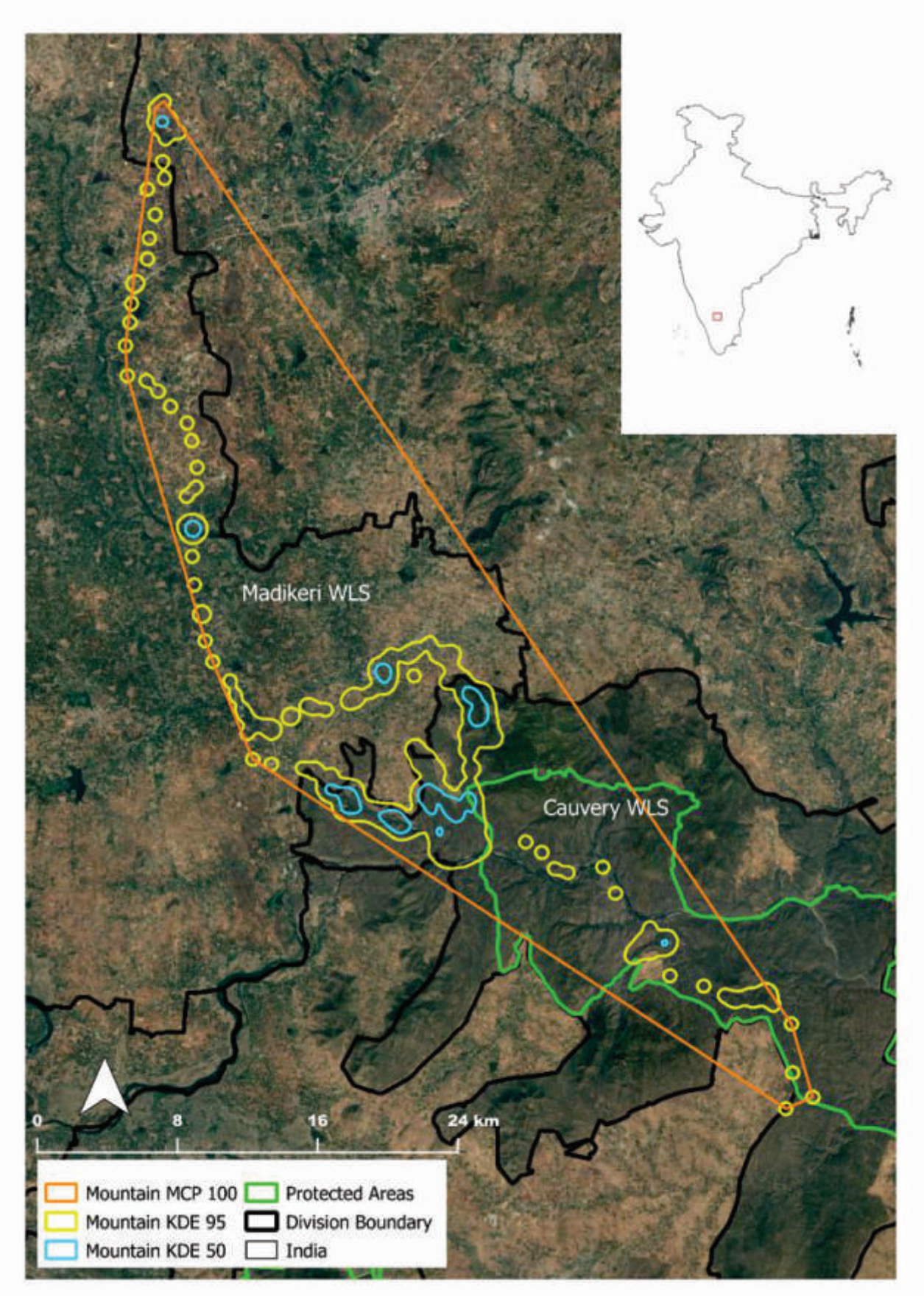


Figure 6.38 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Mountain.*

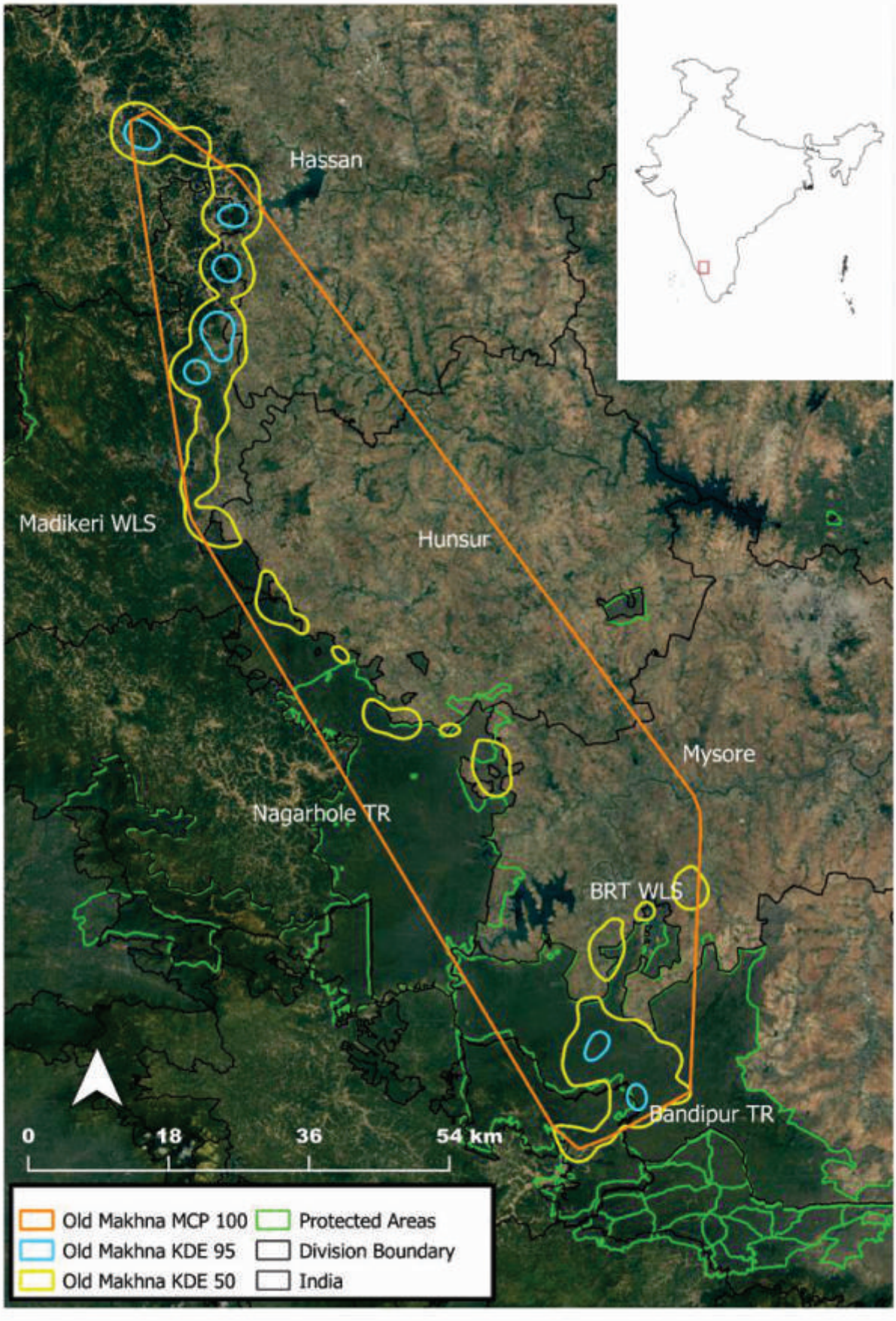


Figure 6.39 : *MCP (100%) and KDE (95% and 50%) for the translocated male elephant Old Makhna.*

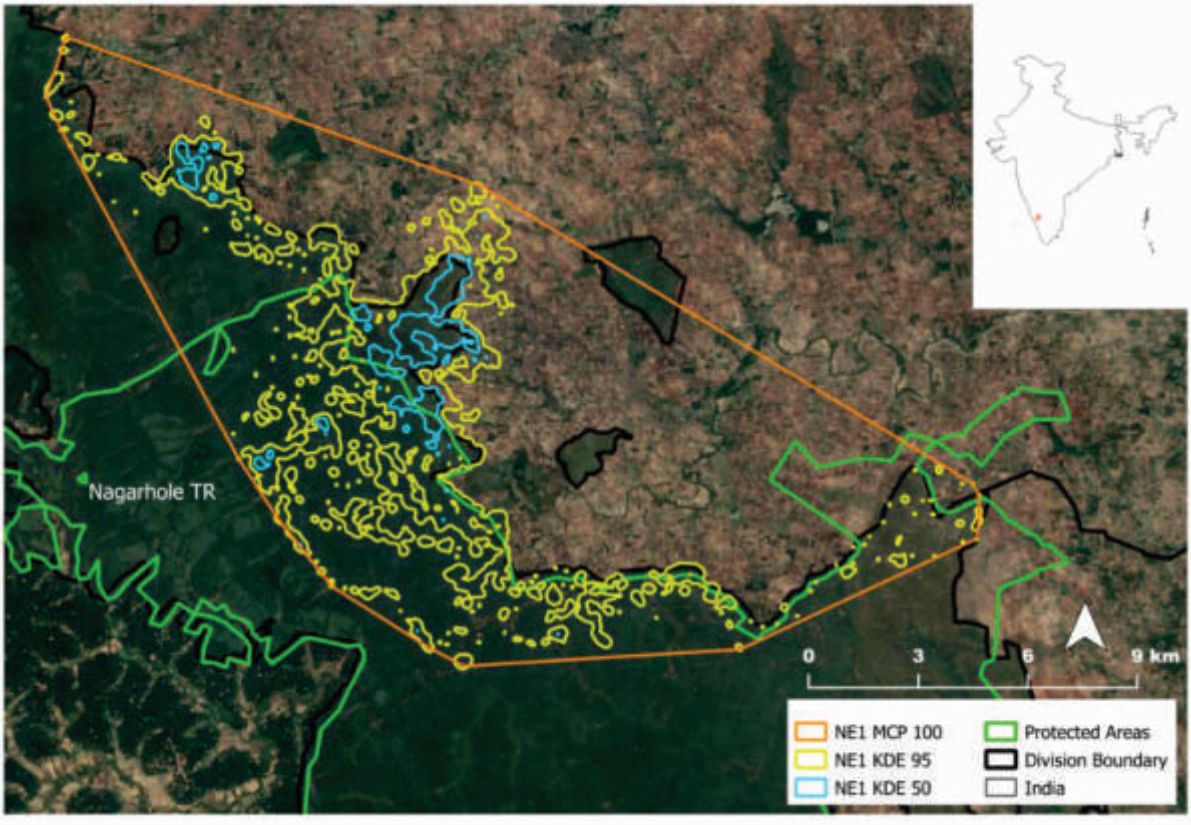


Figure 6.40 : *MCP (100%) and KDE (95% and 50%) for the resident male elephant NE1.*

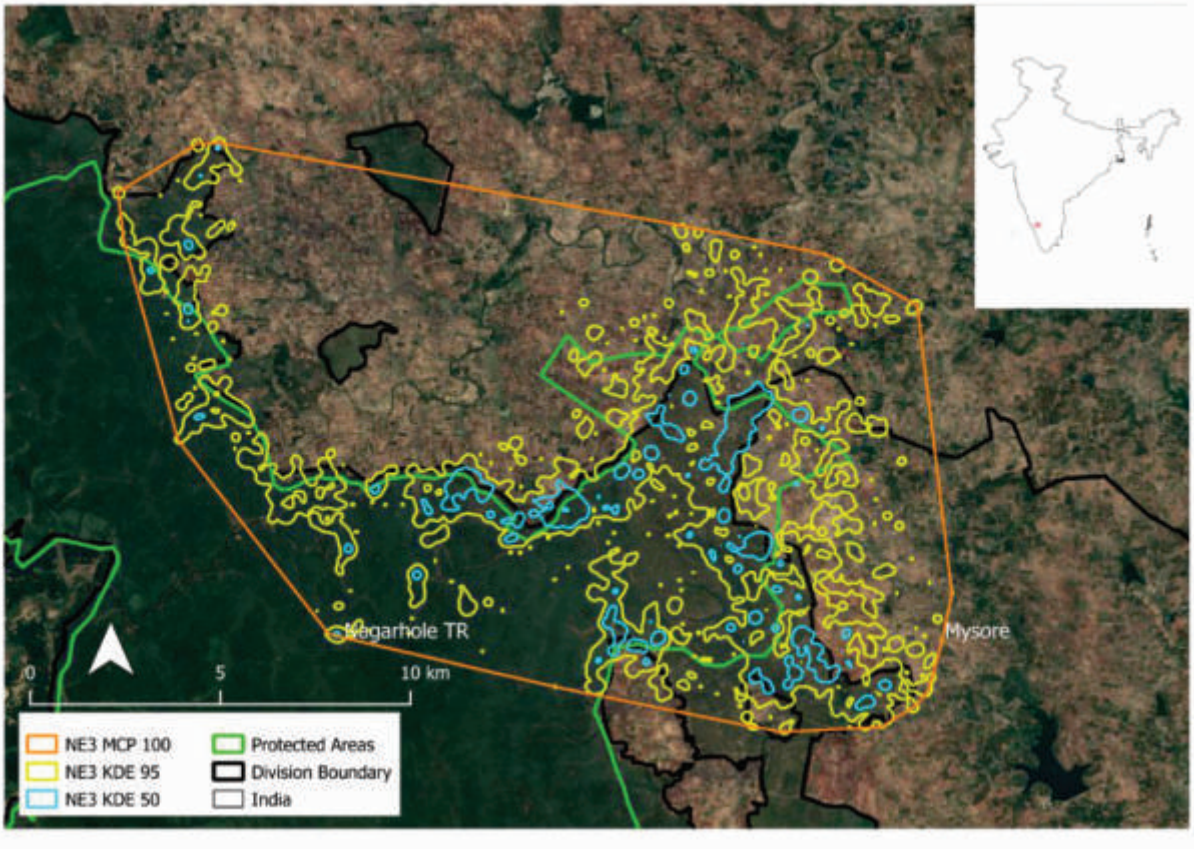


Figure 6.41 : *MCP (100%) and KDE (95% and 50%) for the resident male elephant NE3*

Movement Pattern

The total distance travelled was highest for Chota Bheem (1921.03 km). It was observed to be lowest for Mountain (54.68 km) among the translocated elephant, whereas, for the resident elephants, it was observed to be on an average of 1645.93 km (Table 6.19). The mean for the total recorded daily distance for the nine translocated and two resident collared elephants showed significant differences to exist between them (F=25.97, df=10, p =0.001) (Figure 6.42). The post hoc t-test revealed that the mean distance moved per day by any two elephants was statistically significant (post hoc t-test, p<0.05), except between Colonel and Chota Bheem; Kusha and Chota Bheem; Gunda and CKM; Haveri tusker and CKM; NE1 and CKM; Kusha and Colonel; Haveri tusker and Gunda; NE1 and Gunda; Kusha and Haveri tusker; NE1 and Haveri Tusker (Figure 6.43).

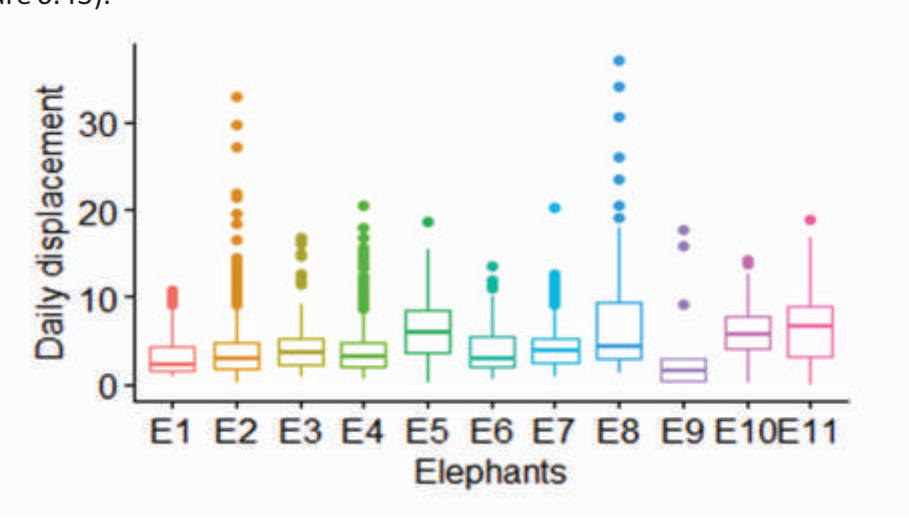


Figure 6.42 : *Average distance travelled per day by translocated elephants in Karnataka (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

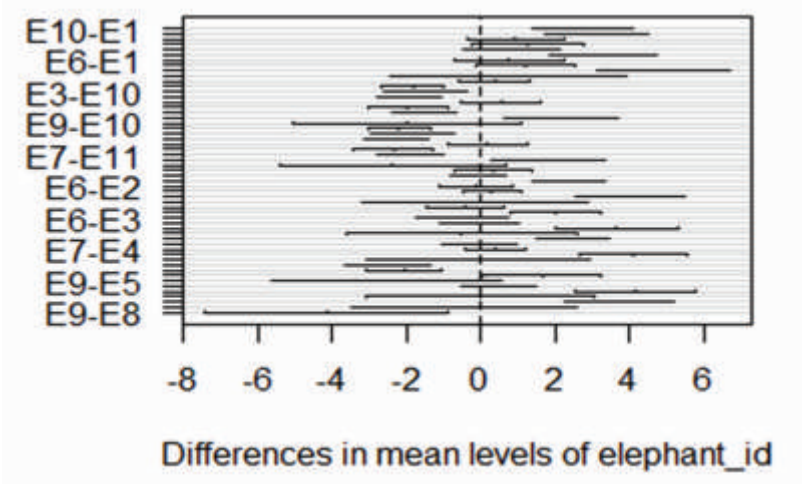


Figure 6.43 : *Differences in the mean levels of daily distance moved by elephants (if an interval does not contain 0, the corresponding means are significantly different. (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8= Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

The mean step length observed among the individuals was statistically significant (F = 146.9, df=10, p=0.001) (Table 6.19; Figure 6.44). No significant difference between the mean step length was observed between Colonel and Chota Bheem; Haveri tusker and CKM; Ne1 and CKM; Haveri tusker and Gunda; NE1 and Gunda and NE1 and Haveri tusker. Statistically significant difference was observed in the mean step length between all the other pairs of elephants (post hoc t-test, p<0.05) (Figure 6.45).

Table 6.19 : Detail movement pattern of translocated elephants in Karnataka.

S. no.	Elephant ID	Total distance covered (km)	Mean daily displacement (km) (±SD)	Mean step length displacement (±SD)	Mean day displacement (±SD)	Mean night displacement (±SD)
1	Chota Bheem	1921.03	4.07(±3.80)	0.18(±0.30)	2.24 (±2.05)	3.28 (±3.31)
2	Colonel	1692.57	3.98(±3.04)	0.18(±0.28)	1.66(±1.80)	2.97(±3.02)
3	Gunda	1158.86	6.43 (±3.53)	0.24 (±0.38)	1.75 (±1.26)	4.68 (±2.90)
4	Haveri tusker	696.67	3.92 (±2.61)	0.17(±0.25)	1.52(±1.18)	2.39(±1.72)
5	Kusha	1587.03	4.37 (±2.47)	0.20(±0.28)	2.05(±1.16)	2.31(±1.75)
6	Mountain	54.68	3.90 (±5.95)	0.71 (±0.76)	2.15 (±2.64)	3.20 (±4.74)
7	CKM	667.98	4.42 (±3.24)	0.19(±0.29)	2.30(±2.18)	2.10 (±1.59)
8	Basava	285.17	3.16 (±2.23)	0.13 (±0.23)	1.56 (±1.21)	1.53 (±1.34)
9	Old Makhna	532.68	8.06 (±8.08)	0.36(±0.63)	2.55 (±3.76)	5.51 (±5.16)
10	NE1	1641.26	5.90 (±2.47)	0.25(±0.33)	1.41 (±1.02)	4.55 (±1.99)
11	NE3	1650.6	6.27 (±4.17)	0.32(±0.45)	1.34 (±1.24)	5.43 (±3.23)

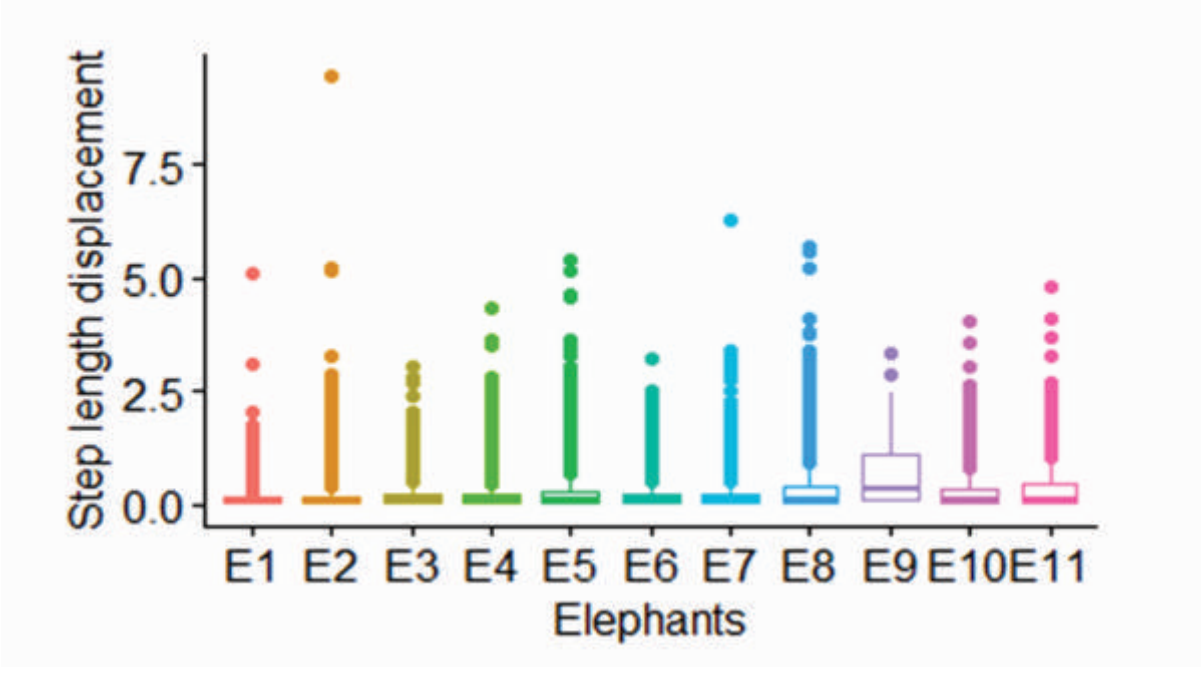


Figure 6.44 : *Average distance covered per relocation (mean step length) by translocated elephants in Karnataka (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

A significant difference was found in the distance travelled by the elephants during the day as well as night (F=10.61, df =10, p=0.001) (Figure 6.46A & 6.47A) and (F=44.24, df=10, p=0.001) respectively (Figure 6.46B & 4.67B; Table 6.19)

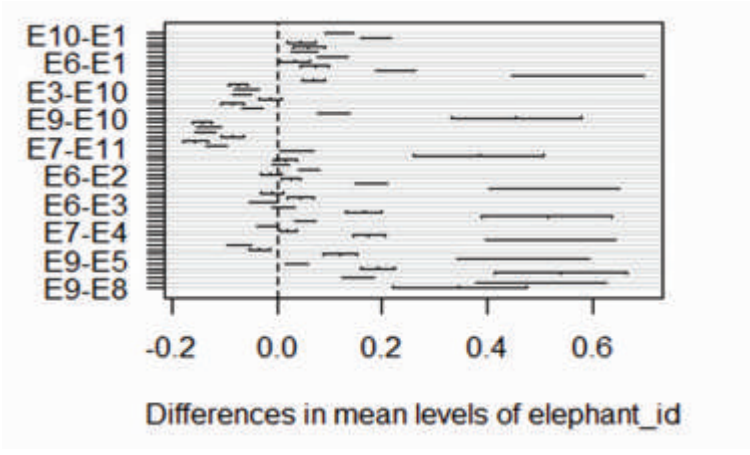


Figure 6.45 : *Differences in the mean levels of distance moved by elephants per relocation (step length) (if an interval does not contain 0, the corresponding means are significantly different (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

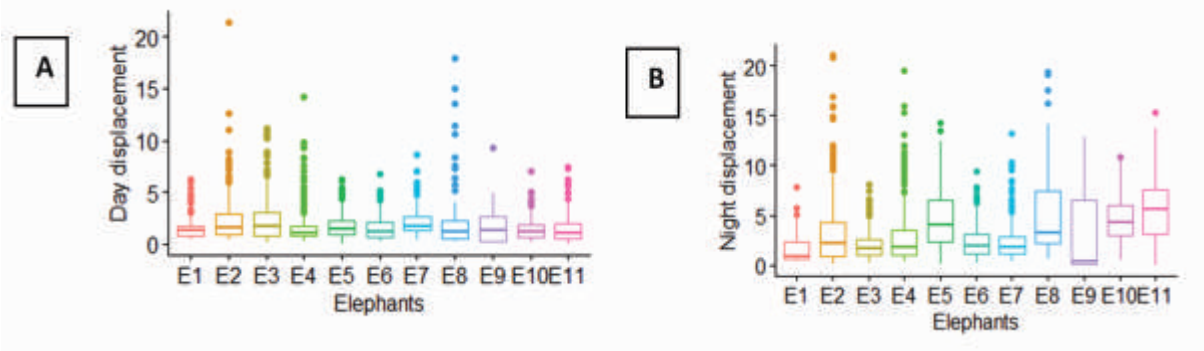


Figure 6.46 : *Average distance travelled during (A) day and (B) night by translocated elephants in Karnataka. (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

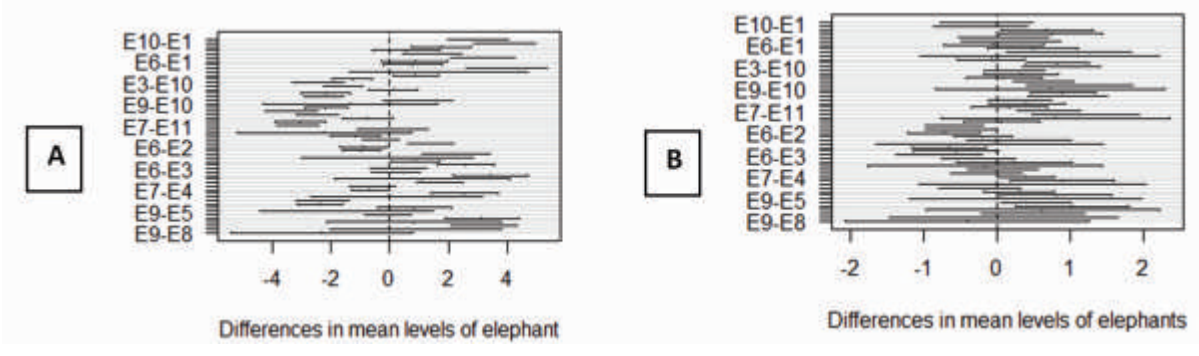


Figure 6.47 : *Differences in the mean levels of distance moved by elephants during (A) day and (B) night (if an interval does not contain 0, the corresponding means are significantly different (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

A significant difference was observed between the mean monthly displacement of the collared elephants (F=2.64, df =10, p=0.0135) (Figure 6.48 & 6.49).

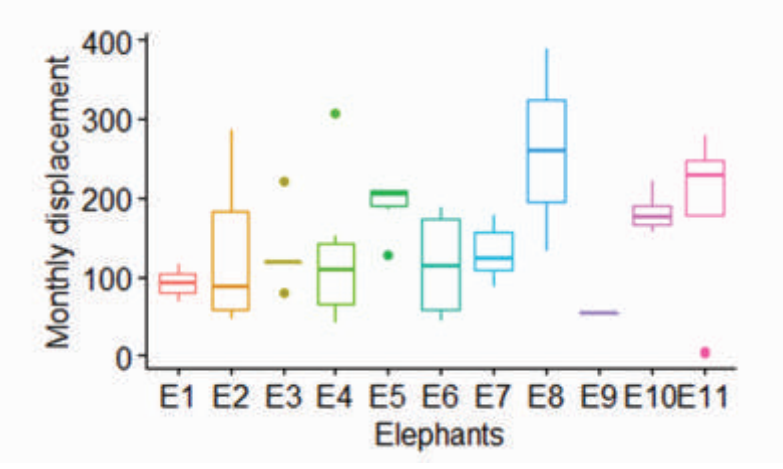


Figure 6.48 : *Average monthly displacement by translocated elephants in Karnataka. (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

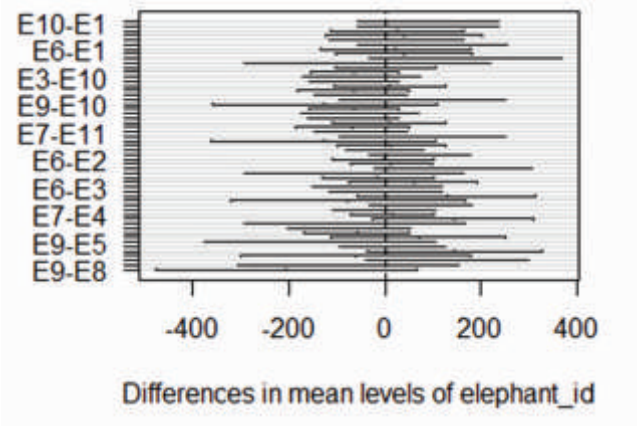


Figure 6.49 : *Differences in the mean levels of distance moved by elephants in months (if an interval does not contain 0, the corresponding means are significantly different (E1= Basava, E2= Chota Bheem, E3=CKM, E4= Colonel, E5= Gunda, E6= Haveri Tusker, E7= Kusha, E8 = Old Makhna, E9= Mountain, E10=NE1, E11= NE3).*

Habitat use and habitat selection

Forest comprised most of the habitat of Basava, Haveri Tusker, Kusha, Colonel, CKM, Gunda, Chota Bheem and NE1, whereas, Crops were predominant in NE3, Old Makhna, Mountain, and Chota Bheem. Scrub/Shrub was dominant in Mountain, Chota Bheem, Gunda, CKM and Colonel. Grassland was present only in Basava, CKM, Colonel, Gunda, Haveri Tusker, Kusha and Old Makhna, though in significantly less proportion. Water was present in all elephant home ranges but less in Colonel and Gunda. Built-up was found in varied ranges in the home ranges of all the male elephants. Flooded vegetation and bare ground comprised a negligible proportion of the male elephants' home ranges (Figure 6.50).

Resource selection function revealed a preferential selection for land use categories by the translocated male elephants (log-likelihood = 198.44, df = 37, p =0) (Figure 6.51) and the resident male elephants (log-likelihood =60.44, df = 8, p < 0.001) (Figure 6.52).

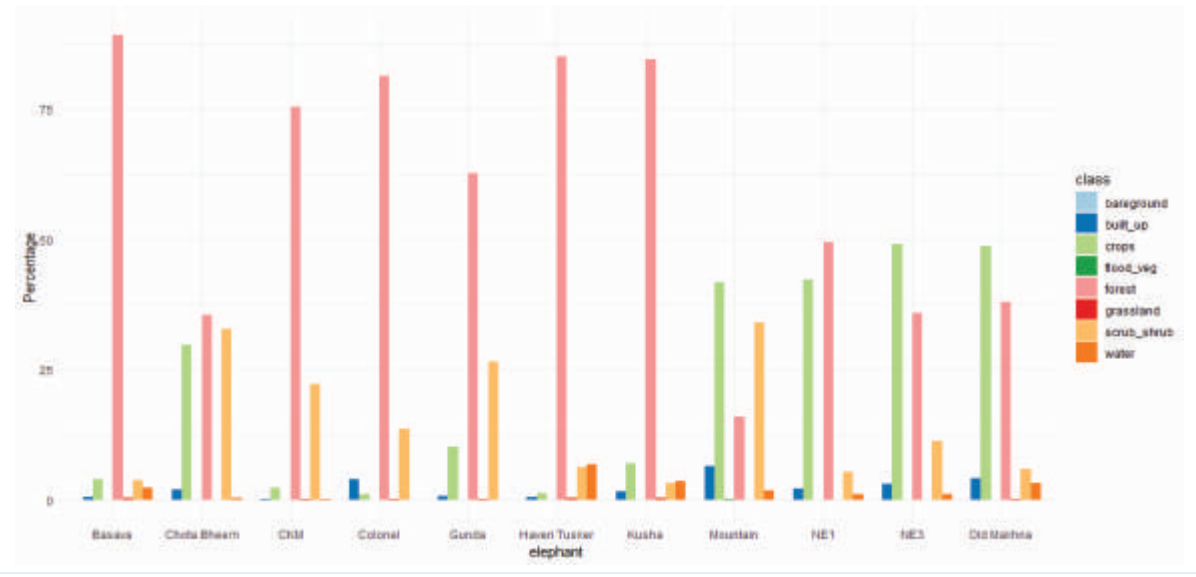


Figure 6.50 : *Habitat composition of translocated male elephants along with two resident elephants (NE1 and NE3).*

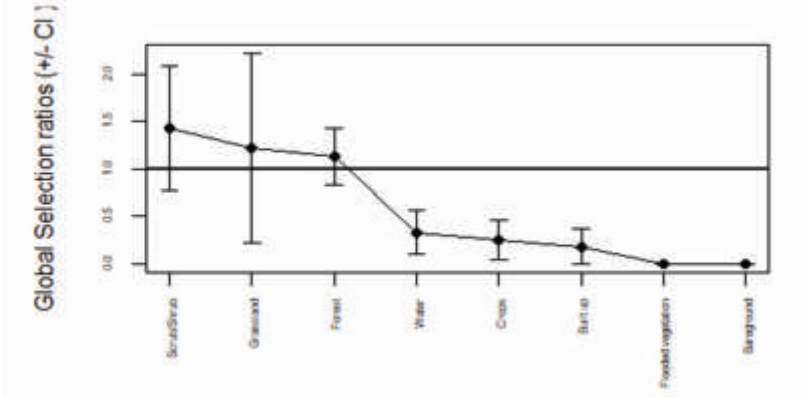


Figure 6.51 : *Land use proportion in relation to availability vs. used based on Manly's test for translocated male.*

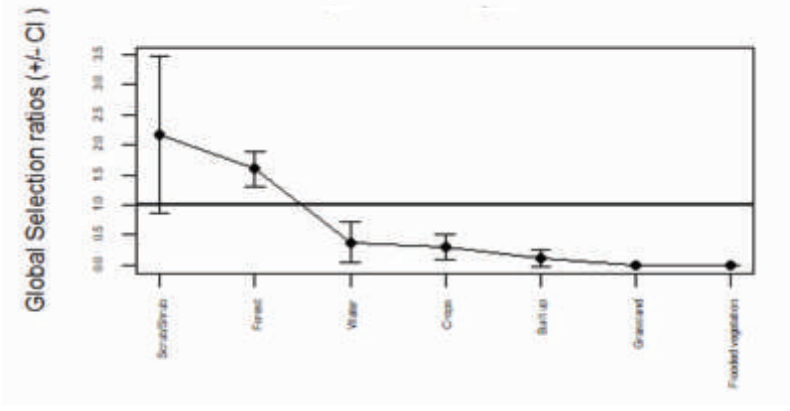


Figure 6.52 : *Land use proportion in relation to availability vs. used based on Manly's test for resident male.*

6.4 Discussion

Elephants, being the largest terrestrial mammals, are of significant interest to researchers who seek to understand their home range patterns and factors that influence them for conservation and management purposes. Ecologically, elephants are known to be adaptable and exhibit remarkable behavioural plasticity. In Asian elephants, there is a sexually dimorphic social organization where adult males live alone while female groups take care of the young. Male-male competition and promiscuous mating are present in this species. Adult male Asian elephants undergo periodic physiological, hormonal, and behavioural changes known as "musth" for 2-3 months each year (Eisenberg et al., 1971). On the other hand, African savannah elephants (*Loxodonta africana*) have been the subject of most of the radio telemetric studies on elephant ranging, which have documented a range of home sizes and patterns. Home range sizes vary depending on the location, ranging from 15 to 52 km² in Lake Manyara (Douglas Hamilton, 1973) to 3059 to 15,422 km² (Viljoen, 1989; Lindeque and Lindeque, 1991) in the Namibian desert. These range sizes are inversely proportional to rainfall and primary productivity (Thouless, 1996). High seasonal variation was observed with smaller dry season and larger rainy season ranges (Rodgers and Elder, 1977; Dunham, 1986; Stokke and du Toit, 2002). However, some areas have a dearth of seasonal variation (Grainger et al., 2005) and larger dry season ranges (De Villiers and Kok, 1997). There is limited knowledge about Asian elephant behaviour in the wild, but it is known that they inhabit dense forests with limited visibility and are nocturnal, likely due to the high incidence of human-elephant conflicts in their range, which has prompted them to modify their behaviour and avoid human interaction.

Here we report on the home range of Asian elephants in Kodagu and Hassan district of Karnataka. We discuss the possible determinants of home range size in Asian elephants. This study provides new insights into Asian elephant ranging. A total of 22 (9 females, 4 resident males, 9 translocated males) elephants have been radio-collared. The home range of resident females is 70.83 (±10.20) sq.km, with a daily movement of 4.09 km (±0.401), resident males are 39.28 (±15.141) sq.km with a daily movement of 4.59 km (±1.214) and translocated males home range is 221.71 (±174.78) sq.km with a daily movement of 4.51km (±1.42).

Table 6.20 : Home range study review on Asian elephants

Method	Study	Location	Home range (sq. km)	Sex	Time (months)	Locations	Habitat
Observation	Easa(1988)	South India, Kerela	124	Herd	12	226	Forest and plantations
			157	Herd	12	200	Forest and plantations
	Sukumar(1989b)	South India, Nilgiris	320	Male	26	12	Dry deciduous forests, grasslands
			215	Male	9	7	Dry deciduous forests, grasslands
			170	Male	20	11	Dry deciduous forests, grasslands
			105	Herd	24	14	Dry deciduous forests, grasslands
			115	Herd	23	15	Dry deciduous forests, grasslands
	Desai(1991)	South India, Mudumalai	200	Male	66	209	Dry deciduous forests, thorn forests
			243	Male	19	103	Dry deciduous forests, thorn forests
			168	Male	51	53	Dry deciduous forests, thorn forests
			232	Herd	69	257	Dry deciduous forests, thorn forests
			111	Herd	61	60	Dry deciduous forests, thorn forests
			266	Herd	57	56	Dry deciduous forests, thorn forests
	Datye and Bhagwat (1995)	North-East India	259	Male	36	41	Fragmented dry deciduous forests
			3343	Male	36	39	Fragmented dry deciduous forests
			4349	Male	36	18	Fragmented dry deciduous forests
			3396	Female	36	31	Fragmented dry deciduous forests
VHF Tracking	Olivier (1978)	Malayasia	38	Male	10	16	Secondary Forests
			32	Male	4	10	Primary Forests
			167	Female	7	17	Secondary Forests
			59	Female	6	16	Primary Forests
	Joshua and Johnsingh(1993)	North-Central India	200	Male	22	469	Sal (dry deciduous) forests

			34	Female	22	277	Sal (dry deciduous) forests
	Baskaran et al.(1993)	South India, Nilgiris	623	Female	24	341	Dry deciduous forests, thorn forests
			530	Female	21	294	Dry deciduous forests, thorn forests
			800	Female	22	106	Dry deciduous forests, thorn forests
			375	Male	15	113	Dry deciduous forests, thorn forests
			211	Male	18	224	Dry deciduous forests, thorn forests
	Fernando et al. (2008)	Srilanka	459	Male	34	94	Semi dry deciduous, thorn forests, grasslands
			176	Female	36	172	Semi dry deciduous, thorn forests, grasslands
			64	Female	34	179	Semi dry deciduous, thorn forests, grasslands
			56	Female	28	52	Semi dry deciduous, thorn forests, grasslands
			185	Female	33	109	Semi dry deciduous, thorn forests, grasslands
			83	Male	11	39	Semi dry deciduous, thorn forests, grasslands
			78	Female	6	141	Semi dry deciduous, thorn forests, grasslands
			92	Male	16	21	Semi dry deciduous, thorn forests, grasslands
			125	Female	16	37	Semi dry deciduous, thorn forests, grasslands
			41	Female	6	169	Semi dry deciduous, thorn forests, grasslands
Satellite	Stuwe et al. (1998)	Malayasia	343	Male	6	43	Rainforests, plantations
			6804	Female	11	41	Rainforests, plantations
	Venkataraman et al. (2005)	West Bengal	179	Male	3	384	Dry deciduous Forests, plantations
	Williams et al. (2008)	North India (Rajaji National Park)	404	Male	24	253	Tropical Moist and Dry deciduous Forest
			188	Male	24	285	Tropical Moist and Dry deciduous Forest
			255	Male	10	123	Tropical Moist and Dry deciduous Forest
			184	Male	21	233	Tropical Moist and Dry deciduous Forest
			327	Female	24	235	Tropical Moist and Dry deciduous Forest
			306	Female	24	211	Tropical Moist and Dry deciduous Forest

The majority of resident females and males except for the NE2 herd mostly stay in the coffee plantations rather than in the protected areas which leads to human-elephant conflict instances. Individual home range size variation was within limits attributable to differences in resource requirement due to body size, sex, reproductive status and sociality. In comparison with other published studies of Asian elephants using radio telemetry, home range sizes recorded from southern India (Baskaran and Desai 1996) and Fernando et al.,2019. were much larger. While home ranges comparable in extent to Sri Lanka were recorded from north-central India (Joshua and Johnsingh 1993) and Malaysia (Olivier 1978), these were based on small sample sizes or short tracking periods hence may not be representative. As Asian elephants' range over a large area and mostly avoid humans, studies based on direct observation are likely to underestimate home range size (Baskaran et al. 1993), precluding direct comparison. Accounting for the inherent bias, larger home ranges in South India can be discerned also from reported observational studies. Home ranges of 3000-4000 sq. km estimated by Datye and Bhagwat (1995) in Bihar-West Bengal, north-east India, are by far the largest reported for Asian elephants, and being based on direct observation, the actual ranges may be even larger. While the extreme ranges in north-east India may be partly explained by migration in response to seasonality, the continued range expansion of the winter ranges observed by Datye and Bhagwat (1995), suggests that it maybe unnatural and more a response to human pressure. The extremely large range of 6804 sq. km observed by Stüwe et al. (1998) for a translocated female is likely to be aberrant and unrepresentative of normal ranging. However, it points to the operation of choice in home range determination of Asian elephants.

The tracked elephants exhibited high annual range fidelity, indicating that these ranges reflected the elephants' typical, established ranging patterns and that they have stable, limited home ranges. Studies in other regions of Asia have also noted great fidelity, to annual ranges in southern India and to summer ranges in northeast India (Baskaran and Desai 1996). (Datye and Bhagwat 1995).

Familiarity with resource supply, distribution, and fluctuation, as well as the dangers and pitfalls in their environment, can help elephant individuals better handle the demands of a stochastic (unpredictable) environment. For long-lived species like elephants, high home range fidelity, or consistent ranging patterns, may increase individual fitness and reflect a crucial survival trait. The lack of significant changes in ranging patterns, between wet and dry seasons, as observed in the tracked elephants, suggests their ability to adapt and thrive in a stochastic environment. This implies that they are able to effectively utilize resources within their home range throughout the year, regardless of seasonal changes. Overall, high home range fidelity may be beneficial for long-lived species like Asian elephants in navigating and thriving in their environment, despite seasonal variations and unpredictable conditions.

The occurrence of geographically distinct seasonal ranges in other Asian elephant populations has been reported by some studies (Sukumar 1989a; Baskaran et al. 1993; Datye and Bhagwat 1995), but not by others (Olivier 1978; Easa 1988; Joshua Fernando et al. 16 and Johnsingh 1993). Therefore, the presence or absence of seasonal ranges and migration in other Asian

elephant populations is unclear. Discrete seasonal ranges may be found where resource availability is temporally partitioned between geographically separate locations, where individuals maximize resource access by migrating between them. Elephants may move between savannah and forest habitats, taking advantage of new grass growth with high protein content in the wet season and switching to browse as the dry season progresses (Sukumar 1989a). The limiting effect of water availability on elephant ranges has been observed in numerous studies of African and Asian elephants (Rodgers and Elder 1977; Dunham 1986; Sukumar 1989a; Stokke and du Toit 2002). Elephants are believed to be restricted to the vicinity of perennial water sources during the dry season and released from such resource reliance with the arrival of rains, which has led to smaller dry season ranges. We did not notice any seasonal variation and the abundance of water reservoirs in our study area are similar to African elephants in Kruger National Park, where the presence of numerous artificial water sources was thought to account for their absence of seasonal variation in ranging (Grainger et al. 2005), water availability may not be an important determinant of elephant ranging patterns in our study area as water is available throught in the landscpe. The tracking data and observations suggested extensive range overlap between members of the same group as well as between different groups, between males and females, and between males.

The overlap of core areas between some of the females from different groups in our study is unusual and may represent highly productive and important feeding areas. Elephants ingest substantial amounts of low-quality food, feeding for about 17 hours a day (Sukumar 1989a) on a wide range of plants (Mueller-Dombois 1972; Ishwaran 1983; Steinheim et al. 2005) to meet their nutritional requirement. Therefore, for Asian elephants, food represents a limiting resource that is dispersed, the exploitation of which necessitates a major investment in time and locomotion. Consequently, the cost of territorial defence is likely to outweigh the benefits accruing from exclusive use of a territory. Access to receptive females is closely related to male reproductive success and may be the limiting factor for polygynous males, therefore male spatial organization may be determined by female distribution (Belcher and Darrant 2004). Home-range overlap rather than male intra-sexual territoriality is probable where males cannot successfully defend multiple females (Sandell 1989; Belcher and Darrant 2004). Ranging patterns have major implications for the management of elephants and mitigation of human-elephant conflict. It is likely that ranging patterns of Asian elephants vary widely in response to the environment. Hence information on local populations is essential for their conservation. Future studies need to be extended and wide geographic study of ranging, across a wide spectrum of resource availability, seasonality and elephant densities, will allow better evaluation of hypotheses on the determinants of ranging patterns.

Translocation of Asian elephants is a widely implemented conservation practice that aims to manage elephant populations and mitigate human-elephant conflicts. This approach involves capturing elephants from areas where conflicts with humans are prevalent and relocating them to suitable habitats with lower human presence. Extensive research has been conducted to assess the efficacy and impact of such translocation programs. Studies, including the work of

Ramesh et al. (2017), have highlighted the primary objective of translocation as the reduction of conflicts arising from human encroachment into elephant habitats. By moving elephants to areas with less human activity, the strategy aims to safeguard both human communities and elephant populations. This proactive approach has proven effective in reducing conflicts and enhancing the coexistence of humans and elephants. Translocation programs consider various factors before implementation. Sukumar (2003) emphasizes the importance of evaluating the size and health of the elephant population, identifying suitable habitats in the destination area, considering genetic considerations to maintain diversity, and understanding the social structure of the elephants being translocated. Careful assessment of these factors ensures the success of the translocation process and the welfare of the elephants involved.

The techniques and protocols used in elephant translocation have also been studied extensively. Fernando et al. (2003) highlights the use of tranquilizers to safely capture and restrain elephants, specialized equipment for transportation, and the necessity of post-release monitoring to assess the adaptation and survival of translocated individuals. These protocols are crucial in minimizing stress and potential risks during the translocation process. Successful translocation programs have demonstrated positive conservation outcomes. Fernando et al. (2008) notes that by reducing conflicts and restoring ecological balance, translocation initiatives contribute to the long-term survival of the Asian elephant species. Moreover, translocation allows elephants to occupy previously uninhabited or depopulated areas, promoting genetic diversity and maintaining healthy populations. While translocation shows promise, it also presents challenges and limitations. Baskaran et al. (2020) point out potential stress-related health issues for translocated elephants, disruptions to social structures, risks of injury or mortality during capture or transportation, and the need for long-term monitoring and management of translocated populations. Addressing these challenges requires continuous research, monitoring, and adaptation of translocation protocols to ensure the well-being and successful integration of translocated elephants. Furthermore, community participation and engagement are integral to the success of translocation programs. Ramesh et al. (2017) emphasizes the importance of involving local communities, raising awareness about elephant conservation, and implementing measures to address human-elephant conflicts. By fostering understanding, cooperation, and support among communities, translocation initiatives can achieve sustainable coexistence between humans and elephants.

In conclusion, through research and comprehensive studies conducted by various experts in the field, the translocation of Asian elephants has emerged as a valuable conservation strategy. It offers an effective means of reducing conflicts, restoring ecological balance, and ensuring the long-term survival of the species. However, careful consideration of various factors, adherence to protocols, ongoing monitoring, and community involvement are critical for the success and sustainability of translocation programs.

SECTION III

Wild pig
(Sus scrofa)
&
Nilgai
(Boselaphus tragocamelus)

Chapter 7

UNDERSTANDING WILD PIG ECOLOGY AND HUMAN-WILD PIG CONFLICT

Mariyam Nasir, Lallianpuii Kawlni, Vishnupriya Kolipakam, Qamar Qureshi

The wild boar is distributed throughout the country except for Jammu & Kashmir, high elevation areas in the Himalayas and desert areas of Gujarat and Rajasthan (Chauhan et al., 2009; Keuling & Leus, 2019; Prater, 1980).

It is an omnivorous ungulate species, is most widely distributed globally, and is known to cause extensive damage to farms adjacent to forests or wooded areas globally (Pandey et al., 20 16). The species is classified as "Least Concern" under the IUCN Red List (Keuling & Leus, 2019). The species is a habitat and food generalist, occurring in various habitats, forming a significant prey base for large carnivores and important scavengers across its range (Ahmed, 1995). Its generalist nature, adaptability in human-impacted landscapes and high reproductive rate make it among the species most prone to conflict. A study conducted in 11 significant reserves in India found that the wild boar (45%) was the most frequently involved in the conflict, followed by the nilgai (28.1%) and the elephant (13.3%) (Chauhan et al., 2009). Wild boar have been reported to harm potatoes, maize, durum wheat, oats, barley, soft wheat, vineyards, hazelnuts, grassland, sunflowers, chestnuts, grain legumes, and vegetables globally (Amici et al., 2012; Chauhan, 2011; Jin et al., 2021). In Uttarakhand, considerable crop losses caused by wild pigs in both the Terai and hilly terrains. About 90% of the local respondents perceived

conflict with wild pigs and ranked them as the most damaging species in Uttarakhand and Madhya Pradesh (Kumar et al., 2017; Pandav et al., 2021; Chauhan et al., 2011; Senthilkumar et al., 2020). Wild boar damaged 2.29 tonnes (2,290 kg) of wheat, which was about 2.6% of the potential yield (Pandav et al., 2021) and it is a significant agriculture pest, which have caused the farmers even to leave the farming practices (Pandey et al., 2019). Other than crop damage, attacks on humans also occur that result in death or injury is also a significant concern (Chauhan, 2011).

In and around protected areas, the farmers employ different crop protection measures to protect their farm from wild pig raids. Ahmed (1991) observed farmers guarding their fields use indigenous noise making systems, fire crackers and even guns to create psychological barriers to physically isolate wild pigs from farms. Chauhan (2009) recommended local protective methods such as, cooperative guarding of matured crops, wire fences with white, flying, flashing ribbons or plastic strips that produce scaring sounds and other frightening devices around the cropland. Due to the extent of monetary losses to the agrarian community and economy, the species was previously declared vermin by states such as Uttarakhand, Kerala and Bihar. At the same time, Goa has requested Government of India to declare the species as vermin in the state (Mongabay, 2021).

Methodology

Several reports of crop raiding by ungulates, with wild pigs as a major concern in India, provide qualitative information on damage caused by animals, economic loss, and people's perceptions of wild animals. However, there is little quantitative analysis on species densities in these areas and crop raiding patterns. Thus, as of a more extensive study on population management of species involved in the human-animal conflict, a preliminary study is being conducted using a combination of camera trapping and interviews with farmers to understand the ungulate-human conflict in the fields around Pench Tiger Reserve, Madhya Pradesh (PTR), in March 2019 and in Dehradun within 5 km radius with Wildlife Institute of India as the centre from March-April 2023. We have also conducted questionnaire surveys here to understand the pattern of Human-Ungulate conflict with particular reference to human-wild pig conflict.

To determine camera trap locations, two approaches are being used. First, we generated 15 and 25 random points along the boundary of PTR and Dehradun around WII, selected those that appeared as farm fields on Google Earth, and chose final locations based on ground-truthing. We also approached fields near these locations, where we spotted farmers at work and fields with younger crops. Based on our interactions with farmers, we deployed seven camera traps along the park's western boundary for 5-7 trap nights and 19 camera traps around Wildlife Institute of India in various villages for 60 trap nights. These cameras were placed at a height of 45 cm from the ground, usually on the edge of the field along a forest boundary, capturing animals as they enter and exit the field. Cameras were set to capture maximum photos at night since crop raiding is known to be highest then. We found two major crop types in Pench Tiger Reserve during this season – wheat and gram (channa) – and placed three cameras in each crop

type and one in a mixed field of wheat and channa. In Dehradun, wheat was the primary crop harvested during the season, along with some fodder crops; hence the cameras were deployed in wheat fields. Detailed questionnaire surveys with closed and open-ended questions were conducted in various villages around the Wildlife Institute of India to get more insights into the human-wild pig conflict. We have conducted the surveys among different age classes, gender and education status of the people. The survey households were selected randomly with agriculture field(s) as the area is fastly developing into a concrete jungle with agriculture fields being converted into plots for construction.

Result

Preliminary analyses from camera trap photos from PTR revealed frequent visitation by the wild pig, chital, nilgai sambar deer, and black-naped hare (Figure 7.2), whereas in Dehradun around WII, wild pig, rhesus macaque, jackal, small Indian civet, Asian palm civet, barking deer, sambar black-naped hare, porcupine, and peacock (figure 7.3) were frequent visitors among the herbivores. In two camera traps, leopards were venturing into Dehradun's agricultural fields. Cattle, dogs, and humans were captured almost every trap night in Dehradun, as the landscape is urban with the forest around it.

Figure 7.1 : *(a) Researcher conducting questionnaire survey (b) camera trap deployment and (c) monitoring of the agricultural fields in Dehradun field site.*

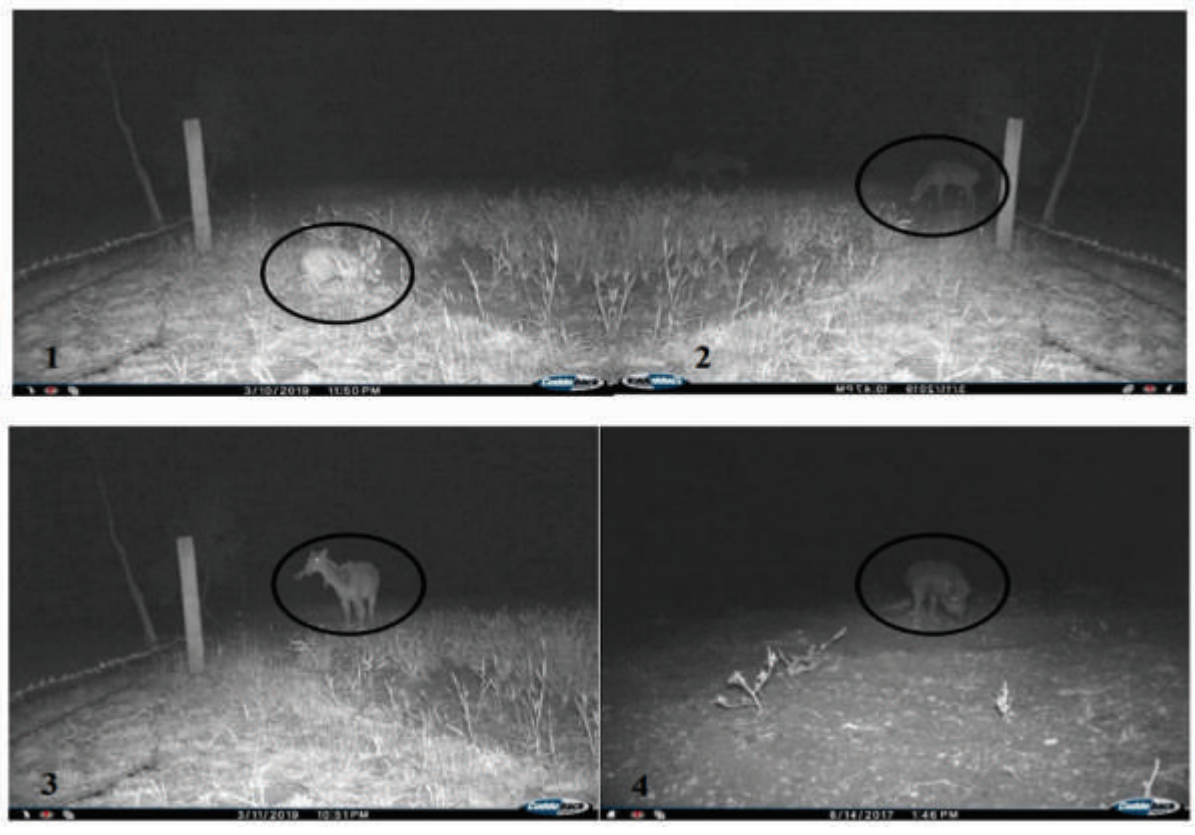


Figure 7.2 : *Camera trap images of 1. Black-naped hare in wheat field, 2,3. Nilgai in wheat field and 4. Wild pig in gram (channa) field at Pench Tiger Reserve.*

Figure 7.3 : *Camera trap images of 1. Leopard, 2. Barking deer, 3. Asian Palm Civet 4. Jackal, 5 Small Indian Civet, 6. Rhesus macaque, 7. Indian grey mongoose, 8. Balck-naped hare, 9. Peafowl, 10. Sambar, 11. Porcupine, 12. Rock pigeon in wheat field, Dehradun.*

The farmers here have also mentioned cattle and dogs as major threats to the agricultural field and wild animals. There was some variation in which species farmers perceived as causing the most damage. However, all farmers reported frustration, especially against wild pigs, as they were hardest to detect at night and caused additional damage by trampling and rooting (figure 7.4) in both the study sites. The following crops to be planted in these fields are maize (corn) in PTR and maize, sugarcane and rice in Dehradun during the monsoon, and farmers reported the most damage by wild pigs during this season and crop type. Due to this, most of Dehradun's farmers have stopped harvesting corn and sugarcane, with corn being almost discarded from agricultural practice. They also complained of increased damage when crops are younger (and more nutritious), soon after planting in December and January for wheat and gram crops and in July and August for maize crops.

Two farmers from the PTR site mentioned crop raiding by gaur, and we found signs (dung, hoofmarks) of gaur near the camera trap in these fields, although no photos of gaur were captured. We found pellets and signs of all reported species in the respective fields.

We have conducted 66 questionnaire surveys from 11 villages around the Wildlife Institute of India. Of the total surveys, 68% of the respondents were males, and 32% were females; 83% belonged to the Hindu community, whereas 17% were Muslim. In Dehradun, the people are mostly doing commercial agriculture on lands taken on lease. In Dehradun, the people are educated or promote education for their children. A section of the respondents was observed to be in service and given their lands on lease to others for agriculture on a 50-50% basis.

Figure 7.4 : *Camera trap images of Wild pig venturing, trampling, feeding and digging in wheat field, Dehradun.*

Additionally, we documented current mitigation methods employed in these sample fields from both study sites. Farmers used various methods, including active guarding with or without dogs, barbed wire, mesh wire, lights, reflectors, and cloth/plastic fladry in both the study sites (Figure 7.5 & 7.6). In Dehradun, the rubble has been tried and tested as an initiative of the Uttarakhand Forest Department to reduce human-wild-pig conflict. All farmers reported that guarding was the most effective, followed by fencing, while lights and reflectors were the least effective at both sites. The rubble wall has been proven very effective, but the exercise was undertaken on a small scale.

Figure 7.5 : *(a) Trampling of wheat crop by wild pig (b) Mitigation methods in gram (channa) field guarding hut, cloth fladryat Pench Tiger Reserve.*

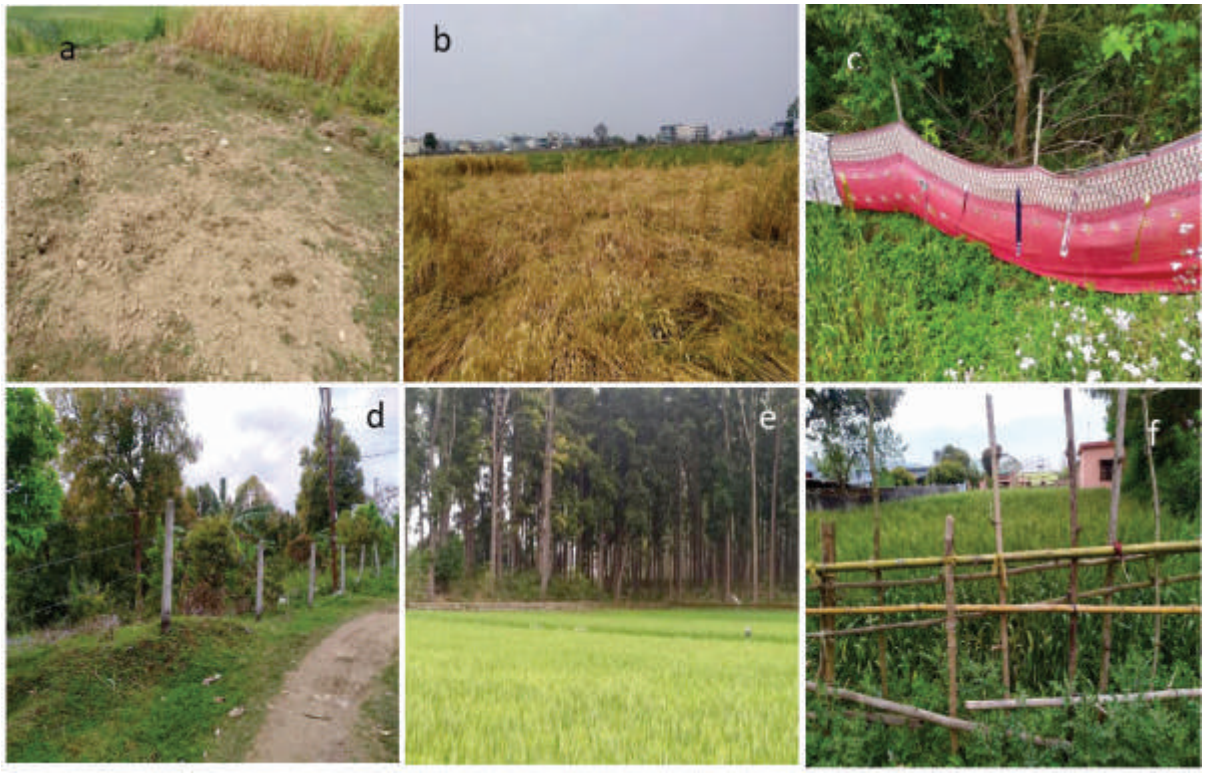


Figure 7.6 : *(a) Digging (b) Trampling of wheat crop by wild pig (c) cloth fladry (d) chain-link fencing, (e) rubble wall (f) wooden fencing as a mitigation method to deter wild pig in Dehradun.*

Chapter 8

UNDERSTANDING NILGAI ECOLOGY AND HUMAN-NILGAI CONFLICT

Chandrapratap Singh Chandel, Lallianpuii Kawlni, Vishnupriya Kolipakam, Qamar Qureshi

8.1 Human-Nilgai Conflict

The blue bull (*Boselaphus tragocamelus*) is a bovid commonly called the nilgai. It is the largest antelope species in Asia (weighing up to 288 kg) with large stature and prominent sexual dimorphism (Prater, 1971; Sheffield et al., 1983). The species is endemic to the Indian subcontinent. The native range of the species in Asia includes the foothills of the Himalayas in Nepal (Dinerstein, 1980), north-eastern Pakistan (Mirza and Khan, 1975), and almost all of India, except eastern Bengal, Assam, east of the Bay of Bengal, and the Malabar Coast (Blanford, 1898; Prater, 1971). Their introduced populations exist in various ranches of south Texas (United States), Mexico and private ranches in the orange free state of South Africa (Sheffield et al., 1983; Lever, 1985; Mathur, 1991). They exploit a diverse range of habitat types. However, relatively open habitats such as scrubland, savanna, and dry deciduous forests at native ranges, whereas root plowed pastures and improved rangelands at introduced ranges are preferred by them (Sheffield et al., 1971; Dinerstein, 1979; Bagchi et al., 2003; Karanth et al., 2009). They are intermediate feeders, and their diet switches from grazing to browsing depending upon the availability of forage (Solanki & Nayak, 1998; Hines, 2016). They have been categorized as a 'least concern species by IUCN, due to their abundant population, wider

distribution, and adaptability to arable lands on the Indian subcontinent (IUCN, 2016). The species has been found to be increasingly involved in road mishaps, human-human conflicts over their population management practices, and attacks on humans in India (Vishnoi, 2016; Khan et al., 2019; Gulati et al., 2021; Gorchiya et al., 2022). The conflict between farmers and nilgai is on the rise due to habitat loss and increased crop damage by the species in the agricultural lands (Bajwa & Chauhan, 2019). The species is known to cause significant damage to agricultural crops, often adjacent to forest patches throughout its distribution in India (Sekhar, 1998; Gubbi et al., 2014; Kumar et al., 2017). Depredation of crops by nilgai herds have been extremely troublesome for farmers in Central and North India particularly in states of Bihar, Uttar Pradesh, Haryana, Gujarat, Madhya Pradesh, Punjab and Rajasthan (Chauhan et al., 2010).

The nilgai though diurnal start raiding crops after dusk (Bayani & Watve, 2016). The movement of the herds through the fields as well using them as shelter when not foraging cause damage to wheat (Triticum aestivum), gram (Cicer arietinum) and mustard (Brassica campestris) crops. This excessive depredation also led to stoppage of the cultivation of sugarcane, pigeon pea, and groundnut crop in some villages of Haryana state, north India (Chopra & Rai, 2009). In 2009 according to the Union agriculture ministry, the extent of crop damage due to nilgai was 50-70% in Uttar Pradesh, 50-60% in Haryana, 10-20% in Gujarat. (Down To Earth, 2016). The changing patterns in agriculture, land use and constant development and population growth of nilgai in these particular areas keep adding up to the human wildlife conflicts all over the country. The government of India has also been providing compensation for the crops that are damaged by nilgai. 3278 cases of crop damage by the species were reported during 2009-13 in Madhya Pradesh and the state government paid the total amount of 1.2 crore rupees as compensation (Babbar et al., 2022). Studies suggest that, they prefer all stages of soyabean, all stages except fruiting of wheat and fruiting stage of chickpea crops (Bayani, 2016). Results of a study at Ramnagar forest division Uttarakhand suggest that in the study area, nilgai were more problematic species than elephants, chitals and sambars because they were raiding croplands located farther from the forest edge (Kumar et al., 2017). During 2013-2018, nilgai were found to be involve in majority (61.70%) of animal vehicle collision incidences in Abohar Wildlife Sanctuary, Fazilka, Punjab. Among different age and sex classes of nilgai, maximum number of animals struck were female, followed by male and yearlings (Khan et al., 2019). Such animal vehicle collision incidences are particularly common in areas where nilgai have higher density

Table 8.1 : A study conducted on agricultural crop damage by nilgai herds based on their density at an area showed the following results (Chauhan, 2011)

Crops	Damages (areas with high nilgai density)	Damages (areas with low nilgai density)
Wheat	35-60%	20-30%
Gram	50-70%	40-55%
Moong	45-70%	40-45%
Guar	-	20-35%
Cotton	-	25-40%

Table 8.2 : Previous studies suggest that following mitigation measures have been adopted to prevent conflict with the species

Mitigation methods	Description	Locations	Notes	References
Guarding	Presence of people to protect resource and chase animals away	India	Moderately effective with 50% decrease in losses.	Bayani et al., 2016
Electric fence	Electric wiring around the resource periphery	India	Effective but cost prohibitive	Karanth & Kudalkar, 2017
Barbed wire fence	Barbed wire fence around the resource	India, Texas	Moderately effective with animals learning to evade barriers over time.	Karanth & Kudalkar, 2017; Foley et al., 2017
Biological fence	Use of thorny shrubs or unpalatable species	India	Moderately effective with habituation over prolonged exposure.	Karanth & Kudalkar, 2017
Chemical Deterrent	Nilgai dung solution, forate insecticide fumes, phenyl solution sprays, tri methyl amine, microbial fermented fish solution	Rajasthan, India and 4 Districts of Nepal	Highly effective, short-term	Meena et al., 2014; Kushwaha, 2022
Visual Deterrent	Scarecrow, fire, lights, lamps	Rajasthan, India	Ineffective, easily habituated	Meena et al., 2014
Noise Deterrent	Drum beating, firecrackers, bells	India	Varying effects, can be habituated	Meena et al. 2014; Ansari 2017
Translocation	Capture, immobilize, translocate	Madhya Pradesh, India	Expensive, labour intensive, shifts conflict to new location, ineffective	MPFD, 2017
Sterilization	Vasectomy	Madhya Pradesh, India	12 males targeted, ineffective, expensive	MPFD, 2017
Culling	Killing of problematic animals	Bihar, India	A total of 2653 animals were culled in the year 2016, could not continue due to socio-political constraints	Hindustan Times, 2017

and thriving in human dominated landscapes. It possesses threat to the life of both human and the animal. There is incidence where the animal attacked a person who was scaring away the animal from his crop land which resulted in the penetrating horn injuries (Gorchiya et al., 2022). Attacks of nilgai are rare due to their timid nature, but there are higher probability of farmers getting injured by the animal while guarding their crops or driving away the animal due to its sheer size and agility.

Socioeconomic questionnaire surveys around Panna Tiger Reserve, Madhya Pradesh

Pilot survey was carried out during 13-23 July 2022. List of villages situated at the 0-5 Km distance intervals from the Panna tiger reserve buffer were obtained from the forest department office. A total of 222 villages were found which belongs to 5 different buffer ranges:

- Panna Range (Panna District)
- Amanganj Range (Panna District)
- Chandranagar Range (Chhatarpur District)

Figure 8.1 : *(a) Interview with villagers (b) Farmer guarding his urad dal crop using field guarding hut*

- Kishangarh Range (Chhatarpur District)
- Madiadau Range (Damoh District)

Maximum number of villages (127) were found to be fall within Panna Range; hence its villages were chosen for the pilot survey. Villager's interviews were conducted in randomly selected villages. Sampled villages were dominated by Yadav and Gond communities which are predominantly agro-pastoralists. People practicing agriculture ranked wild pig and nilgai as a top crop raider, while those practicing livestock rearing blamed tiger and leopard for majority of their livestock losses. Farmers were found to be cultivating til (Sesamum indicum), urad dal (Vigna mungo) and ground nut (Arachis hypogaea) in monsoon season. They were found to be using range of mitigation measures from stone fence and wire fence to night guarding through their field guarding huts.

We also visited villages of Chhatarpur district which fall outside protected area networks. Here farmers were cultivating urad dal, they blamed nilgai as the sole crop raider. Farmers claimed that there are more than 200 nilgai antelopes which live at the adjacent reserve forest (7-8 acres) and frequently damage their crops. We found direct and indirect evidences of crop damage by nilgai. We sighted 1 adult male and 4 females over there.

Figure 8.2 : *(a) Hoof marks of nilgai on crop field (b) Direct sighting of a nilgai male near crop field.*

Based upon our preliminary survey around Panna tiger reserve and due to the unavailability of secondary data on crop damage locations, with the help of Arc-GIS tools we clipped 2 Km of buffer around the boundary of reserve. Villages were chosen based upon their proximity and distance in between. Similarly, villages falling inside the core of the reserve, were also chosen using Arc-GIS tools. In this way a total of 37 villages were identified, situated all around the reserve. Semi-structured questionnaire survey was conducted with farmers in these villages. Households were selected randomly targeting 15-20 households in each village. Questions were asked addressing type of crops cultivated in different seasons, type of species involved in crop damage, percentage of damage, frequency and time of visit, herd size, mitigation measures adopted, whether compensation received and perception of farmers towards these species. A total of 19 villages were covered in this way. The total numbers of households covered during survey were 310, as depicted in the table below. The questionnaire survey revealed that majority of farmers cultivate crops in two seasons namely monsoon and summer. In monsoon farmers mainly cultivate til (*Sesamum indicum*) and urad dal (*Vigna mungo*). Other crops which are grown in monsoon season are ground nut (*Arachis hypogaea*) and paddy (*Oryza sativa*) which is subject to the availability of irrigation facility. In winter season farmers cultivate wheat (*Triticum aestivum*) chickpea (*Cicer arietinum*) and mustard (*Brassica compestris*).

Currently the data obtained through this interview is being analysed to identify the hot spot of human ungulate conflicts and design a more extensive study at the site, targeting variation in both crop type and crop maturity age during both planting seasons. It is further envisaged to estimate the density of nilgai through camera trapping and drone survey in and around fields to gain a thorough understanding of conflict along the boundary of reserve. This will further supplement the existing knowledge on focal species of the project, and help design the protocols for population management using immune-contraception in the study landscape.

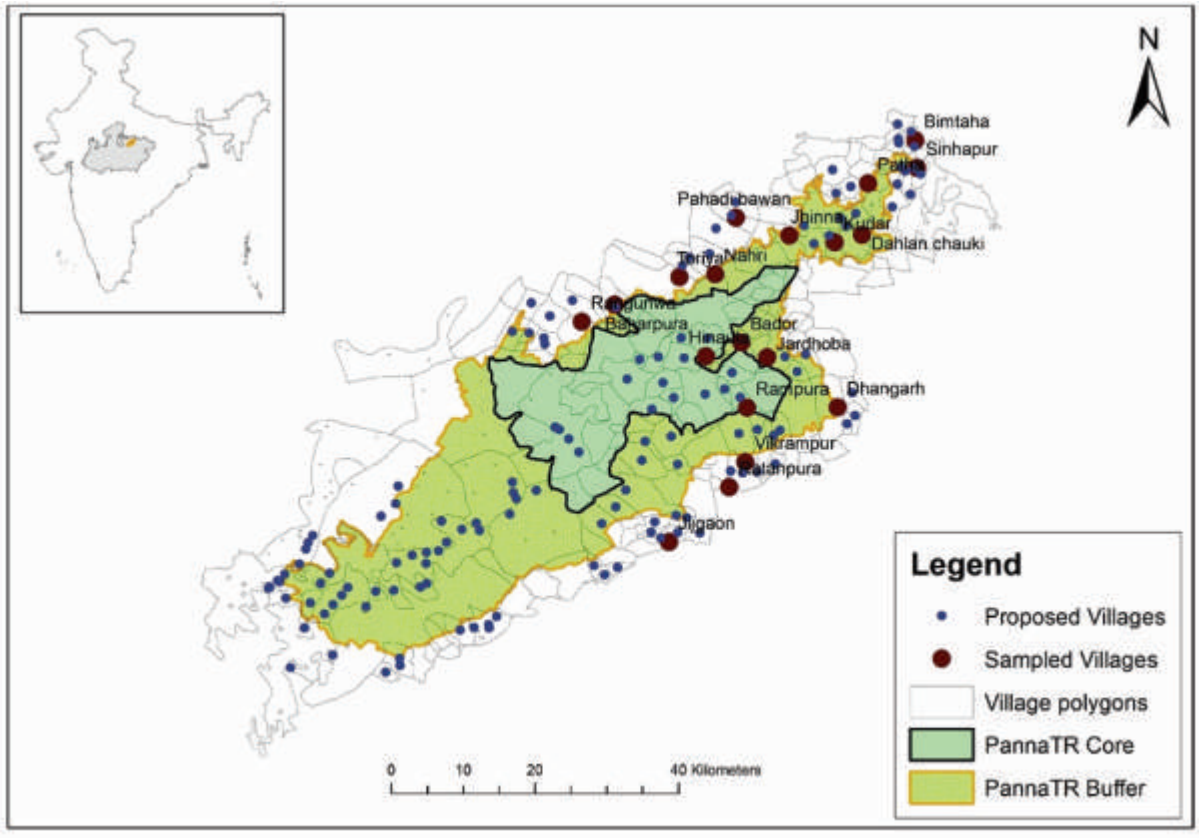


Figure 8.3 : *Map of Panna tiger reserve depicting sampled villages around 2 Km periphery of the reserve.*

Table 8.3 : Villages and respective households sampled during questionnaire survey

S. No.	Village names	Number of households	Number of household practicing agriculture	Number of Households Interviewed
1	Jijgaon	200	200	16
2	Rangunwa	400	350	17
3	Baharpura	300	300	16
4	Rampura	50	40	15
5	Vikrampur	250	250	16
6	Ratanpura	300	250	16
7	Pahadi bawan	800	600	16
8	Toriya	600	500	16
9	Hinauta	700	500	20
10	Bador	60	50	18
11	Patha	150	150	15
12	Bimtaha	100	80	16
13	Sinhapur	1250	1000	15
14	Nahri	50	40	15
15	Dhangarh	80	75	17
16	Jardhoba	280	280	16
17	Jhinna	300	300	16
18	Dahlan chauki	500	400	18
19	Kudar	250	200	16

Results

Semi-structured questionnaire survey was analysed for 200 farmers from different households of 12 villages, practicing agriculture in and around 2Km periphery of the tiger reserve's boundary (Figure 8.3 & Table 8.3). Following findings were obtained:

1) Cropping pattern throughout the year

It was found that due to the scarcity of irrigation facilities, farmers cultivate in monsoon and winter seasons only. In monsoon season 87 (43.24%) respondents cultivate sesame, while 65 (32.43%) respondents were found to be cultivating black gram. Remaining respondents were cultivating 16 (8.11%) green gram, ground nut 11 (5.41%) paddy 11 (5.41%), pigeon pea 5 (2.7%) and sorghum 5 (2.7%). This shows that sesame and black gram are the two major crops in monsoon season.

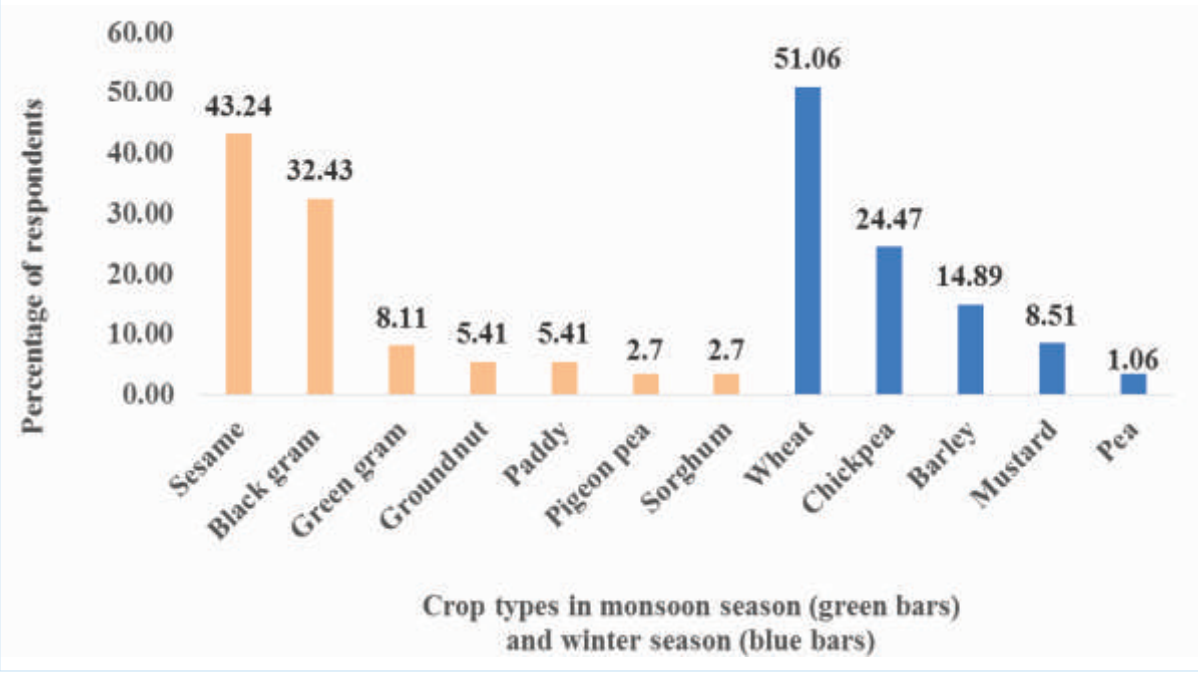


Figure 8.4 : *Percentage of respondents with crop types throughout the year.*

In winter season 102 respondents (51.06%) informed that they cultivate wheat, while 49 respondents (24.47%) named chickpea. Other crops which were cultivated in this season includes barley 30 (14.89%), mustard 17 (8.51%) and pea 2 (1.06%). Hence wheat and chickpea have been found to be two major crops in winter season.

2) Species involved in monsoon season crop raiding

A total of 12 species were found to be involve in crop raiding, out of which 52 (26.15%) respondents named wild pig, while 45 (22.31%) respondents named nilgai to be the major crop raider. Other ungulate species which were named include chital, chinkara and sambar. Langur

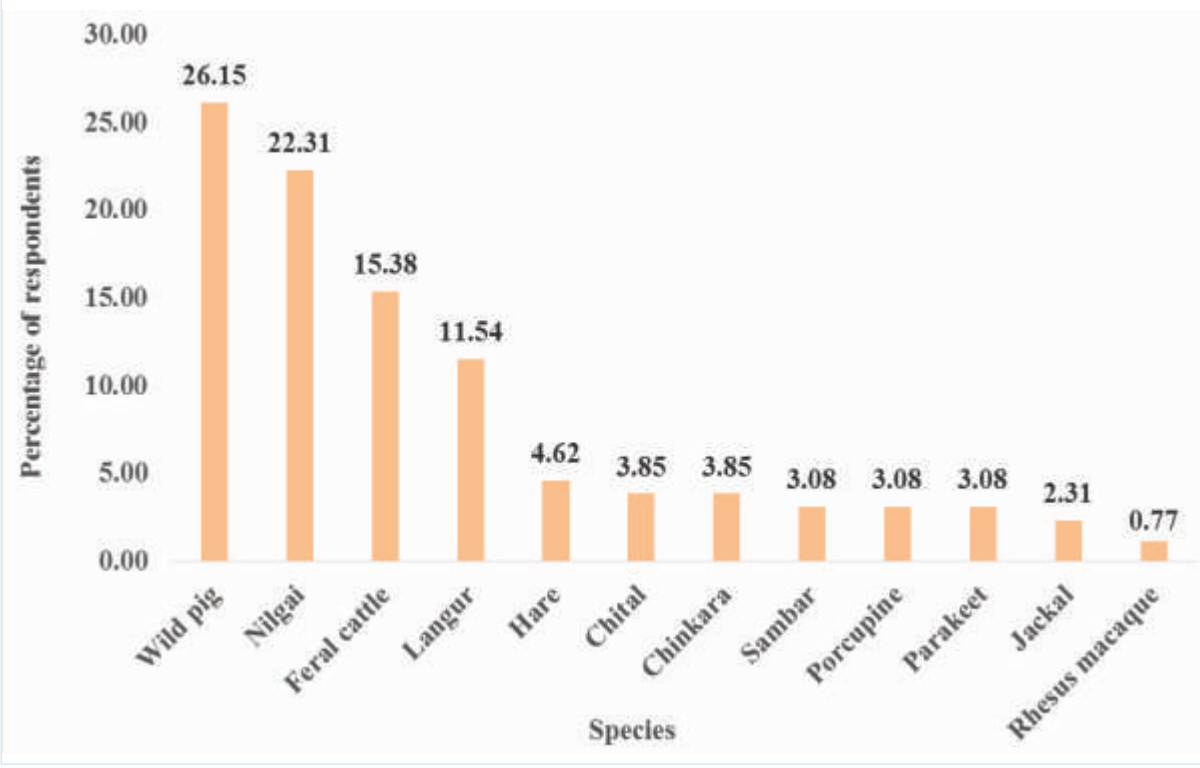


Figure 8.5 : *Percentage of respondents reported different species as crop raider in monsoon season.*

(11.54% respondent) were found to be more problematic over rhesus macaque (0.77% respondents).

Feral cattle (15.38% respondents) were named by many, recent study by Gupta and Krishnamurthy (2021) suggest that there are an estimated 5,600 of feral cattle exist in the core of the reserve.

3) Species involved in winter season crop raiding

A total of 13 species were found to be involve in winter season crop raiding, out of which 40 (19.95%) respondents blamed wild pig and 39 (19.46%) respondents named nilgai for majority of their crop loss in this season. Feral cattle ranked third with 33 (16.30%) respondents perceived them as major crop raider. According to 0.73% of respondents, sloth bear was also found to be involve in crop raiding.

We visited affected farms and found direct and indirect evidences of crop raiding by wild pig, nilgai, langur, feral cattle, and parakeet. Respondents blamed Langur and macaque for their roof tiles damage. All farmers reported frustration especially against wild pig as they were hardest to detect at night and caused additional damage by trampling and rooting.

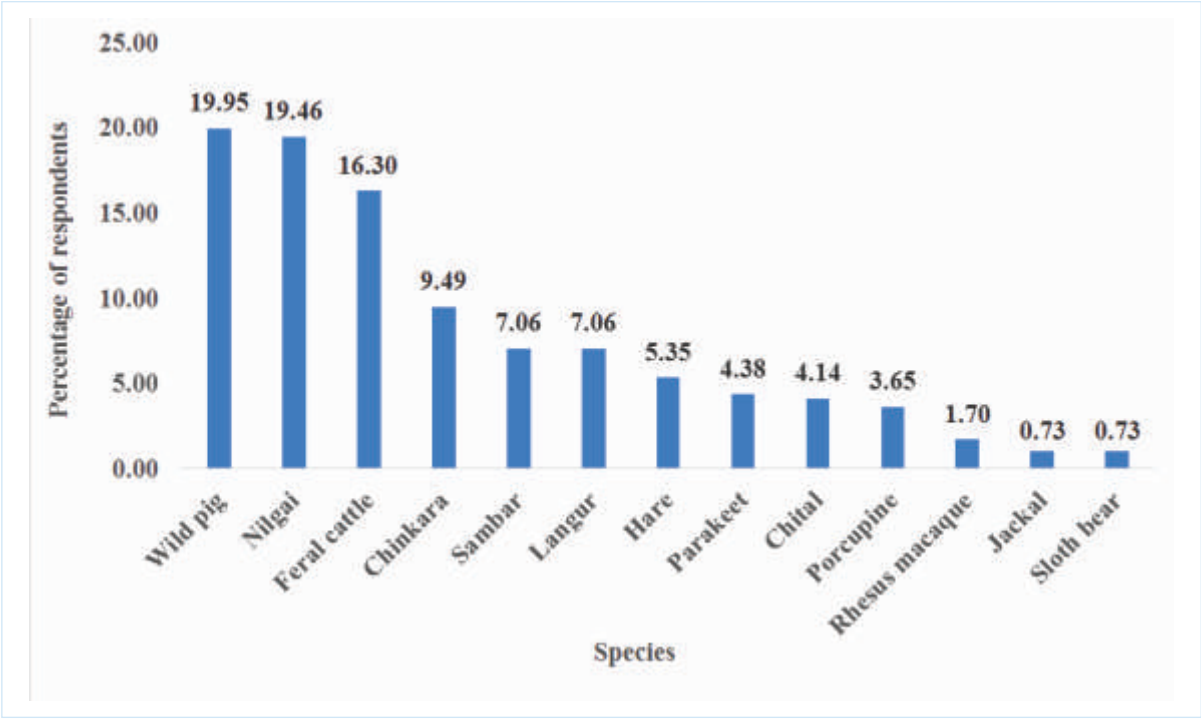


Figure 8.6 : *Percentage of respondents reported different species as crop raider in winter season*

Figure 8.7 : *Sightings and signs of wildlife and feral cattle around croplands (a) Nilgai (b) Wild pig hoof marks (c) Hanuman langur and (d) Feral cattle.*

4) Frequency of farm visit by nilgai and other wild animals

Among different species wild pig and nilgai were found to be most frequent ungulates in visiting croplands. 188 (94%) respondents reported that nilgai daily visit their farm, while 8 (4%) respondents suggested that they visit their cropland 1-2 times in a week. 4 respondents (2%) informed that they sight nilgai 3-4 times in a week at their farm.

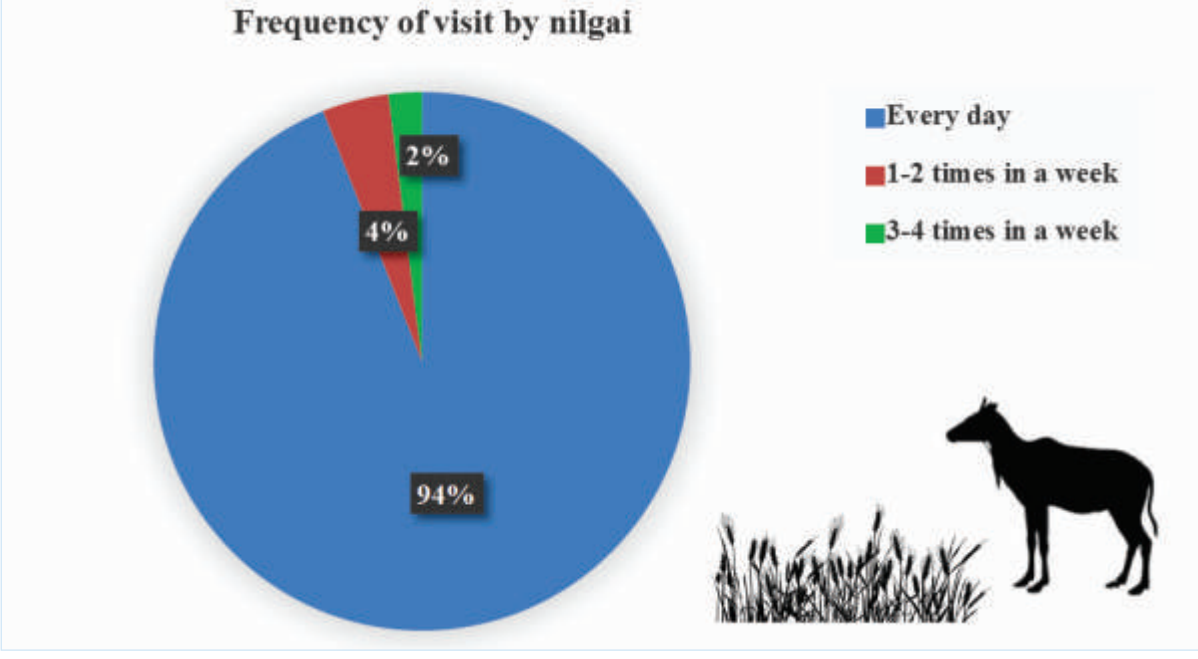


Figure 8.8 : *Frequency of farm visit by nilgai as reported by respondents.*

In the case of wild pig, 196 (98%) respondents reported that they daily visit their cropland. Only 4 (2%) respondents reported that they visit their farm 3-4 times in a week.

5) Herd Size of Wild animals entering the farm

According to respondents, they have spotted solitary as well as herd of up to 45 nilgai. 71 (35.29%) respondents said that they frequently sight groups of 1-5 animals. The second most common herd size was found to be 6-10 animals as reported by 63 (31.37%) respondents.

In the case of wild pig raid their herd size varies from solitary to 100 individuals. Majority of respondents 86 (43.14%) informed that herd size between 21-30 animals were most frequently sighted.

6) Preventive measures being adopted by respondents

It was found that farmers adopt a range of mitigation measures to prevent crop raiding by wild animals. It includes fencing, night guarding by hut and machan. A maximum of 68 (34.01%) respondents informed that night guarding is the most common preventive measure they adopt. 53 (26.53%) respondents have suggested that barbed wire fencing is effective and they

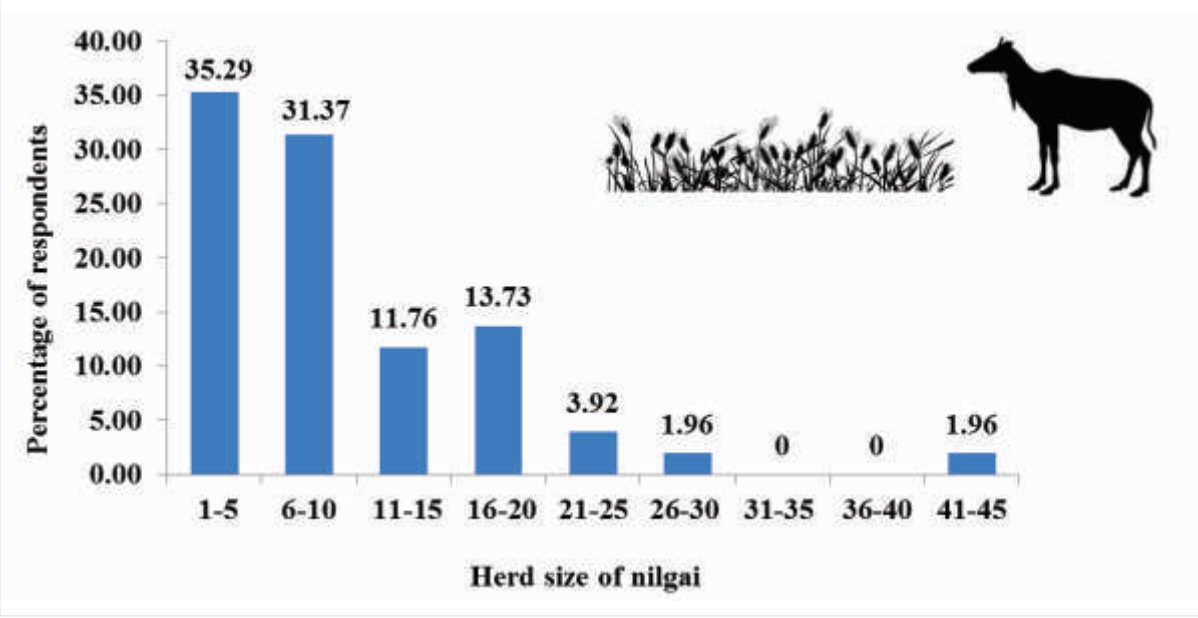


Figure 8.9 : *Herd size of nilgai in the farm as reported by respondents.*

are applying it around their farms. However due to the associated cost many small-scale farmers cannot afford it and hence they depend upon night guarding.

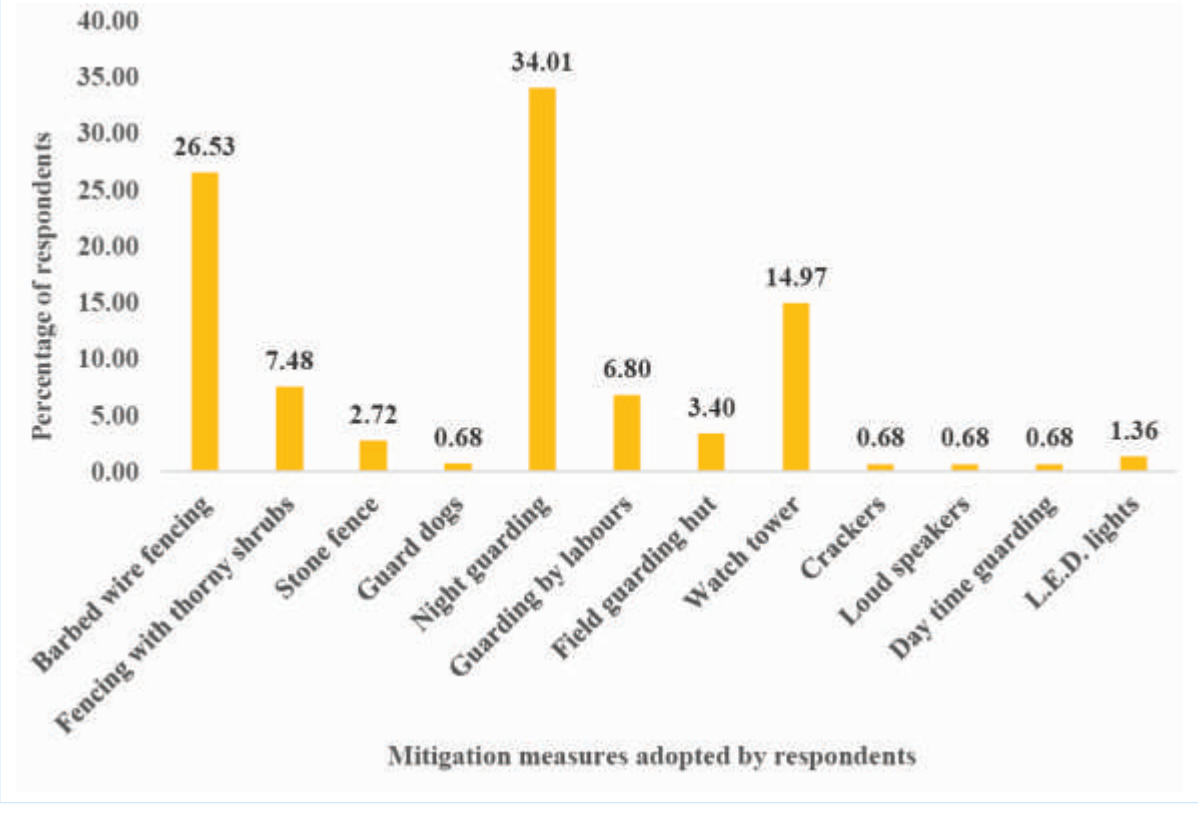


Figure 8.10 : *Different mitigation measures reported by respondents.*

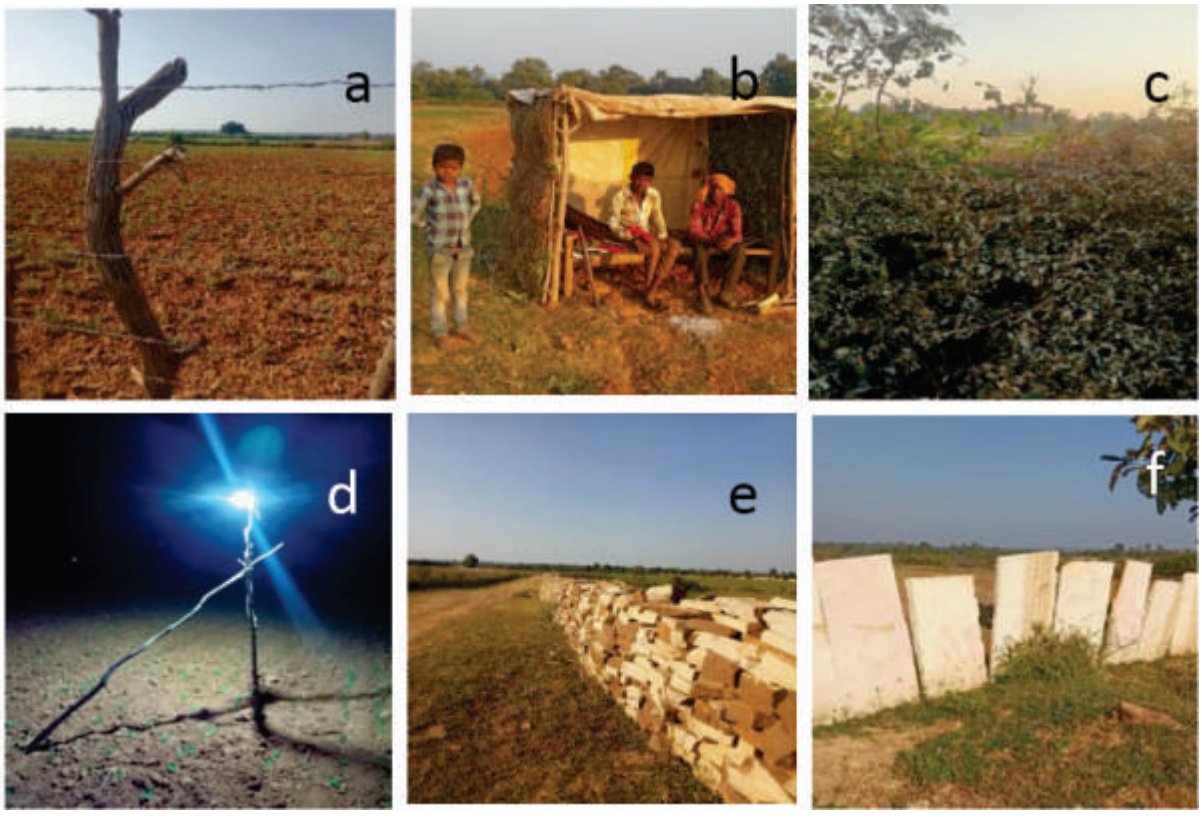


Figure 8.11 : *Crop guarding methods practiced around Panna TR, Madhya Pradesh (a) Barbed-wire fencing (b) Night guarding (c) Thorny shrub fencing (d) LED light (e & f) Different type of stone fencing*

7) Awareness on Compensation

137 out of 200 respondents (68.52%) were unaware about the scheme, while others were familiar with the scheme only. They do not know how and where to claim for the compensation. Only 26 out of 200 respondents (12.96%) said that they are aware regarding the scheme and procedures. Respondent said that joint inspection has rarely been carried out and patwari (local revenue inspectors) do not inspect their affected farms and sometimes ask for bribe, due to this complexity of procedure and multiplicity of departments/authorities, farmers were reluctant to claim for their loss. In the entire process of verification and payment the role of forest department is negligible and hence they do not have any secondary data on crop damage by wildlife.

8) Population trend of nilgai and wild pig as perceived by respondents

189 out of 200 respondents (94.44%) believe that nilgai population is increasing. Similarly, 196 out of 200 respondents (98.15%) reported that wild pig population is increasing. Respondents suggested that the population of nilgai and wild pig is increasing due to prohibition of hunting and higher birth rate. Very few respondents (5.56%) believe that nilgai population has been remain stable over the time. In the case of wild pig only 1.85% respondent reported that their population has been remain stable.

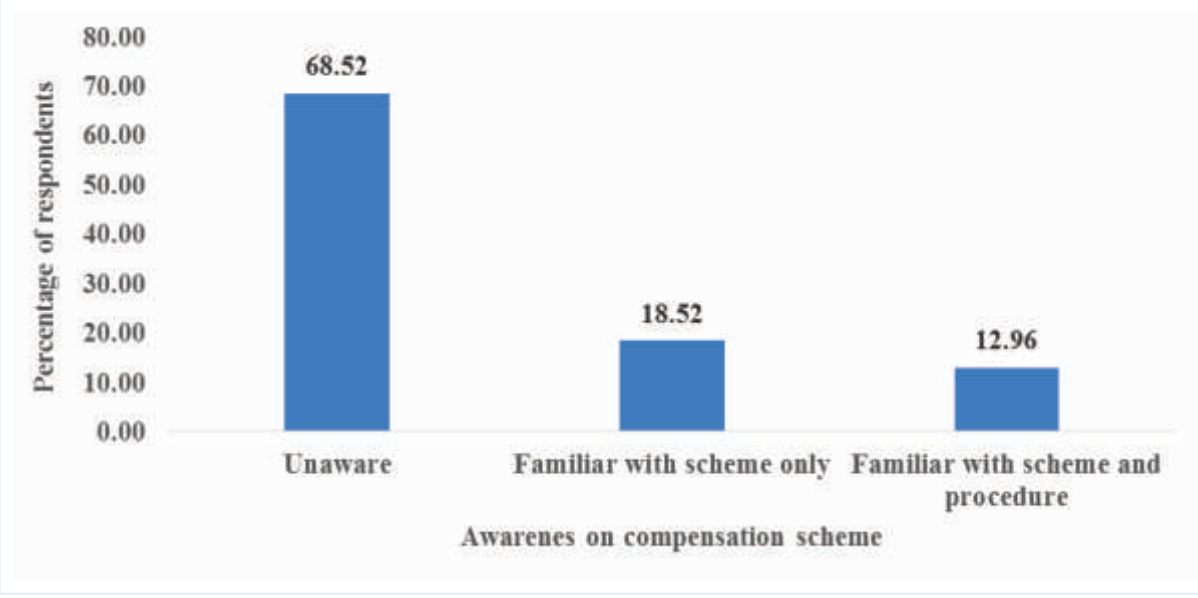


Figure 8.12 : *Respondents' awareness on compensation scheme*

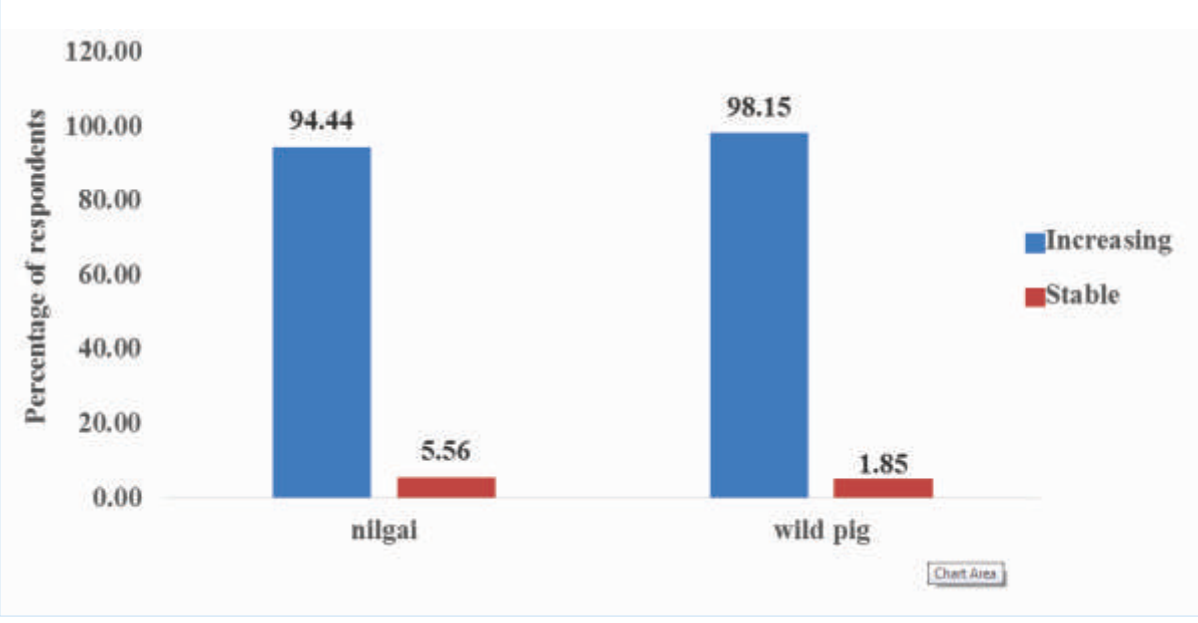


Figure 8.13 : *Population trend of nilgai and wild pig as perceived by respondents.*

8.2 Review of Human-Nilgai conflict reported in print media

Human-wildlife conflict (HWC) can be defined as an interaction where the needs and behavior of wildlife impact negatively on the goals of humans or when the goals of humans negatively impact the needs of wildlife, which may involve damage & cost on either side (Madden, 2004). Due to its social and biogeographical characteristics, developing regions of the world, such as South and Southeast Asia, are particularly vulnerable to this issue (Anand & Radhakrishna, 2017). Wild ungulates have been found to be increasingly involved in raiding crops, damaging properties, attacks on humans, vehicle collisions, competition with and transmission of

diseases to livestock, causing human-ungulate conflict across the globe (Chauhan et al., 2009; Kuemmerle et al., 2011; Acevedo et al., 2014; Duarte et al., 2015; Colino-rabanal et al., 2018; Gross et al., 2018). Overabundant populations of wild ungulates have become a primary concern from management perspectives (Morellet et al., 2007; Cromsigt et al., 2013).

The concept of ungulate overabundance is associated with their negative interaction with humans as well as the environment in which they thrive (Carpio et al., 2021). Whether native or introduced, overabundant populations of wild ungulates are now widespread and have frequently been coming into conflict with humans (Côté et al., 2004; Walter et al, 2010; Keiter & Beasley, 2017). With the introduction of the Wildlife Protection Act (1972) and its associated management actions, coupled with incompatible land use practices, different species of wild ungulates have become overabundant in India, causing persistent negative interactions with humans (Chauhan & Singh, 1990; Chauhan et al., 2009; Bajwa & Chauhan, 2019a).

Nilgai, also known as blue bull (*Boselaphus tragocamelus*, Pallas, 1766), is one such conflict-prone ungulate species in India (Shekhar, 1998; Chhangani et al., 2008; Kumar et al., 2017; Bajwa & Chauhan, 2019b). Although the species is widely distributed in India (Karanth et al., 2009), there is a scarcity of knowledge on their conflict distribution range in the country. Very few studies have attempted to answer this question (Chauhan et al., 2010; Chauhan, 2011). The species is well-known as a crop pest in India (Chauhan & Singh, 1990; Goyal & Rajpurohit, 2000). However, the interactions of their different populations with different crop types over multiple scales have been largely unknown. In this review, we attempted to answer this question through a systematic survey of newspapers to identify the hot spot of conflict. We identified the crop types more vulnerable to nilgai raids than others. We have identified the list of tehsils where population control measures for the species can be considered in the future.

The present study is based on print media coverage of human-nilgai negative interactions at different tehsils of Himachal Pradesh, Uttarakhand, Punjab, Haryana, Delhi (Union Territory), Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Telangana, Uttar Pradesh, Bihar, Jharkhand, and Odisha states in India. Based on their geological zones and geographical locations, these states can be broadly classified into Shivalik hill landscape (Himachal Pradesh, Punjab, Haryana, Uttarakhand, and Uttar Pradesh), Gangetic plain landscape (Uttarakhand, Uttar Pradesh, Bihar). Western Indian landscape (Rajasthan and Gujarat), Central Indian landscape (Madhya Pradesh, Maharashtra, Jharkhand, and Odisha), and Eastern ghat landscape (Telangana and Odisha) (Jhala et al., 2008). The landscapes of these states harbour a unique and rich assemblage of flora and fauna, and have 25 out of 50 tiger reserves in India (Jhala et al., 2019). The area has biogeographic zones ranging from Western Himalayas, Punjab and Gangetic Plains in the north, Desert and Semi-arid areas in the west, Deccan Peninsula in the south and Eastern highlands in the east (Menon, 2014).

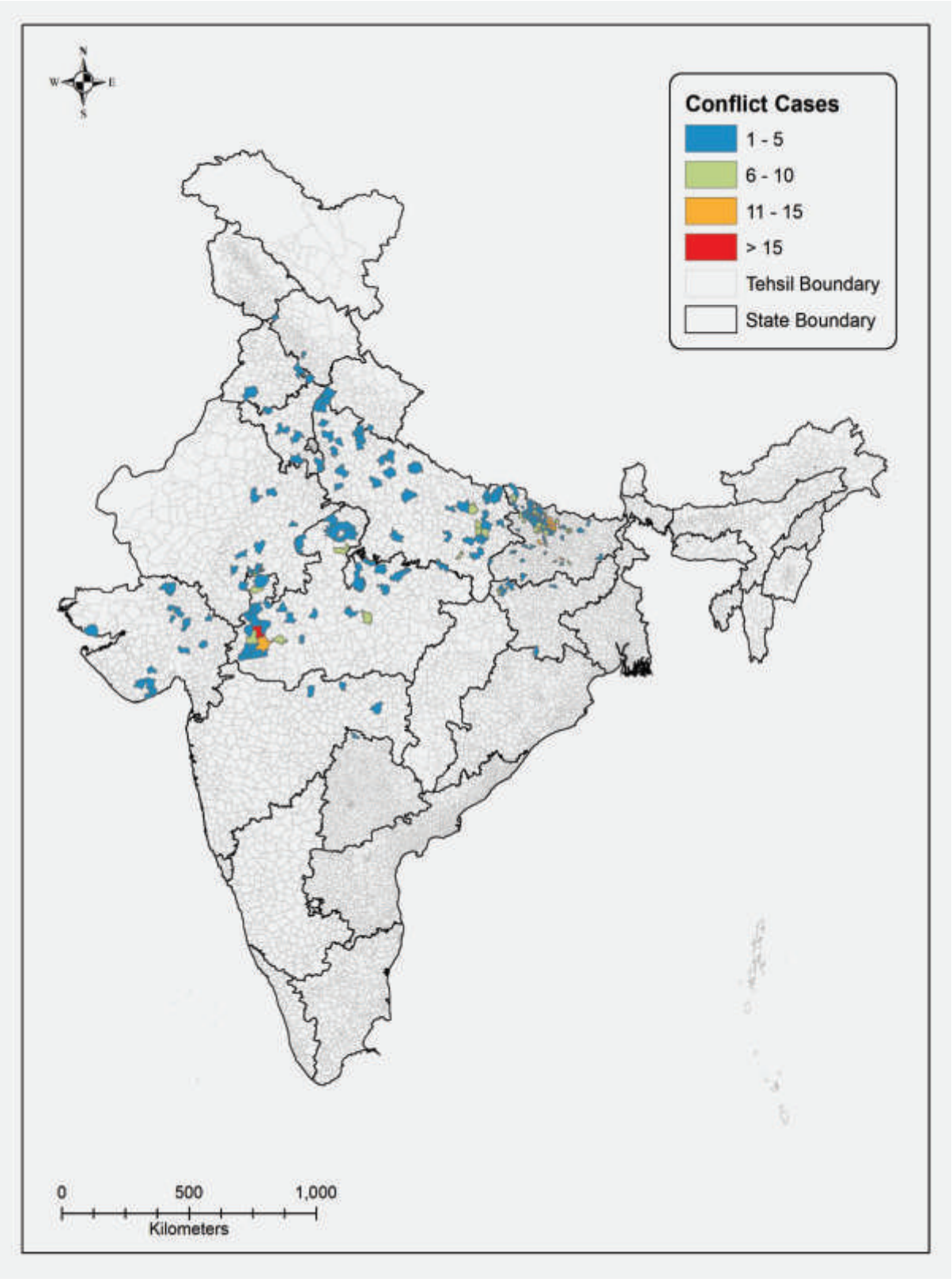


Figure 8.14 : *Distribution of human-nilgai conflict cases at the tehsil level based on newspaper reports.*

Methods

The secondary data on human-nilgai negative interactions in India is obtained through a systematic survey of news articles from 2018-2022, for a total duration of 5 years (Alexander & Quinn, 2008; Athreya et al., 2015). We mainly considered English and Hindi-language-based newspapers for review. We used English and Hindi keywords along with 'nilgai' or 'blue bull' and searched in the news section of the Google search engine till the last tab. After screening for language and time frame, we extracted information on types of negative interactions and location of conflict at the state, district, and tehsil levels. In this way, data were extracted from online editions of 23 publications, including 10 English newspapers and 13 Hindi newspapers. We recorded all the crop types affected by nilgai. In cases where the news article did not specify the crop name or type, we assigned them to an unspecified crop category. If different types of conflict were mentioned in a single news report, each type was considered unique and recorded multiple times (Athreya et al., 2015). In cases where we could not obtain data at the tehsil level, the district name is searched along with previous keywords. The conflict type was broadly categorised into crop raiding, vehicle collisions, attacks on humans and other types of conflict. Here other types of conflict include a potential threat to the safety of both humans and nilgai, for example, the presence of free-ranging nilgai in airways.

Since we found that different nilgai populations have affected over 40 types of crops in India, we broadly classified them into cereals, pulses, oil-yielding crops, vegetables, and other cash crops categories. The relative frequency of each crop type affected was estimated in percentage for each crop type by summing up the total cases of damage for that crop type and dividing it by the sum of all crop types, and then multiplying it by 100 to get the relative frequency in percentage.

Similarly, the sum of each conflict category is divided by the sum of all the conflict categories and multiplied by 100 to obtain the relative frequency of a conflict type. For spatial mapping of conflict, tehsil-level information was used. We obtained the ESRI website's GIS database at the tehsil level for India. A hot spot map at the tehsil and state level was generated by summing all types of conflict categories, including different crop types, and using it as the number of conflict cases at the tehsil level. Based on the sum of the number of affected tehsils, state level and country-level hot spot map of conflict was generated.

Results

A sum of 679 conflict cases was recorded from 368 unique news reports from 209 tehsils of 13 states and 1 union territory in a time frame of 2018-2022 by different newspapers. However, the number of conflict cases in each of these tehsils and states suggests that its severity is different across them.

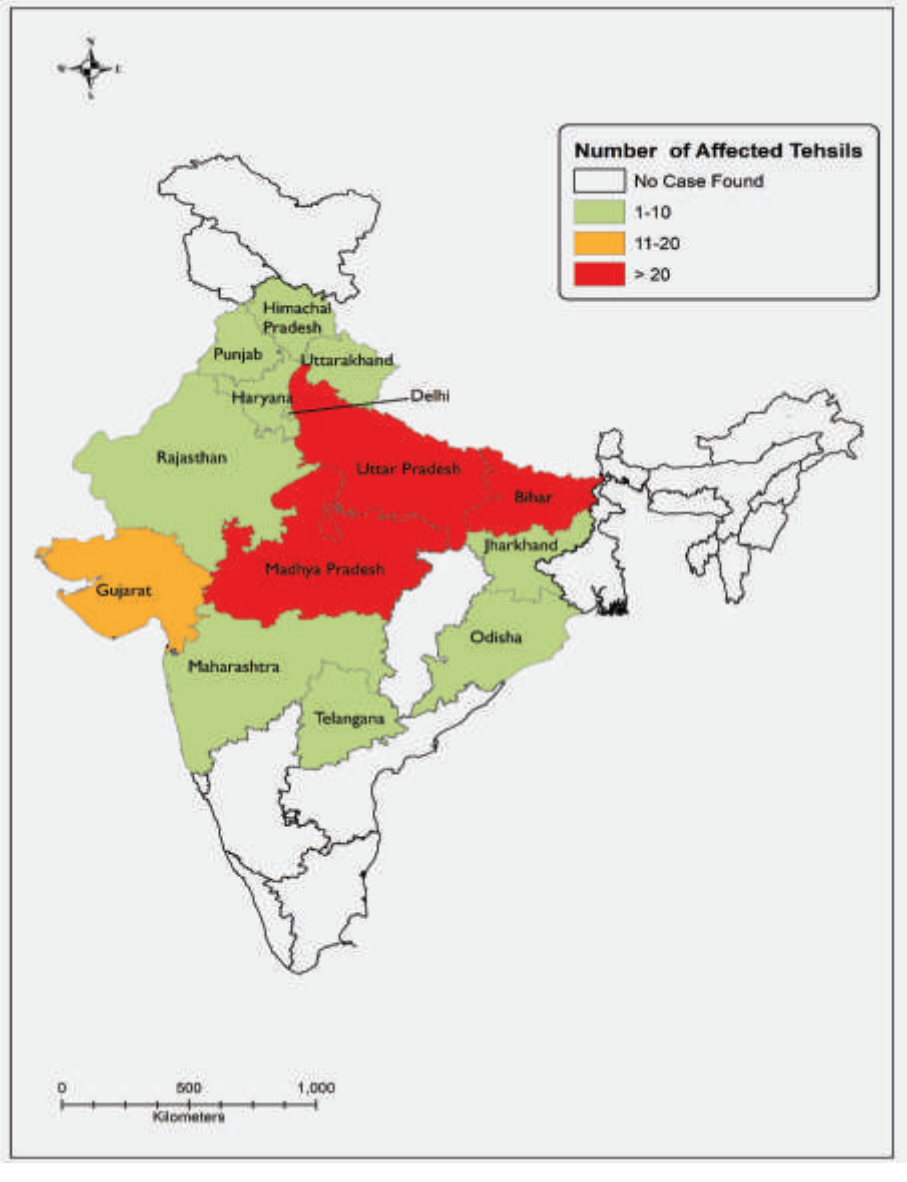


Figure 8.15 : *Distribution of human-nilgai conflict at the state level based on newspaper reports*

Table 8.4 : Number of conflict cases in each tehsil of respective states and union territory

Affected States	Affected Tehsils	Number of Conflicts Cases
Bihar	78	313
Delhi (Union Territory)	2	2
Gujarat	16	17
Haryana	7	11
Himachal Pradesh	1	1
Jharkhand	6	28
Madhya Pradesh	40	154
Maharashtra	3	3
Odisha	1	2
Punjab	4	4
Rajasthan	7	8
Telangana	1	1
Uttar Pradesh	42	134
Uttarakhand	1	1
Grand Total	209	679

Relative frequency of different conflict categories

Out of these 368 news reports, the maximum number was reported for crop damage (76.63%, 282 reports) and vehicle collisions (19.02%, 70 reports), followed by other conflicts (2.45%, 9 reports) and attacks on humans (1.9%, 7 reports).

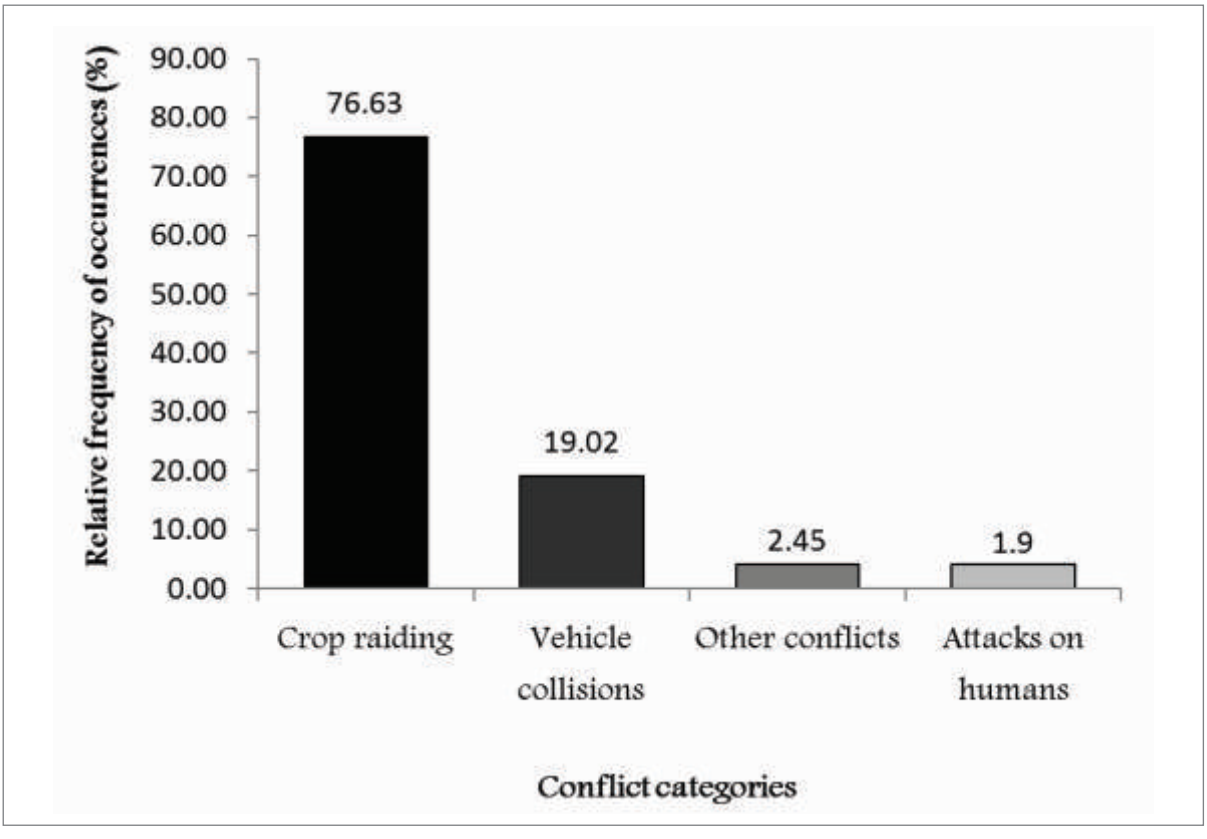


Figure 8.16 : *Relative frequency (%) of occurrence of different conflict categories*

Relative frequency of different crops raided by nilgai

It was found that among different cereal crop categories, the maximum number of crop raiding was for wheat (9.48%) and maize (9.36%), followed by paddy (4.55%), sorghum (0.13%) and pearl millet (0.13%). Among different pulse crop categories, pea (4.55%), pigeon pea (4.42%) and chickpea (3.79%) were found to be at higher risk of nilgai raid. Mustard (3.41%) and linseed (1.64%) were two major oil yielding crops raided by nilgai.

Among different cash crop categories, sugarcane (3.16%) and opium (1.64%) were more vulnerable. It was found that nilgai raided 18 types of vegetable crops.

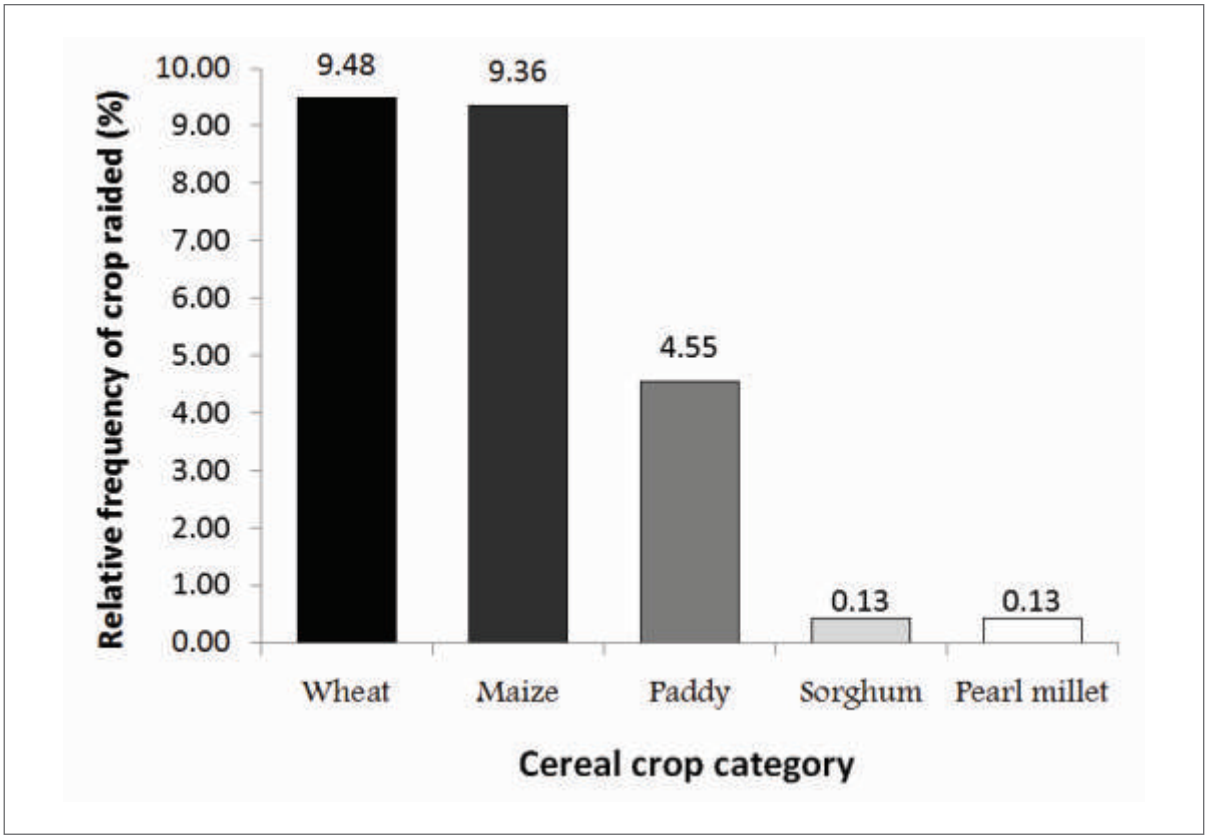


Figure 8.17 : *Relative frequency (%) of cereal crop categories raided by nilgai.*

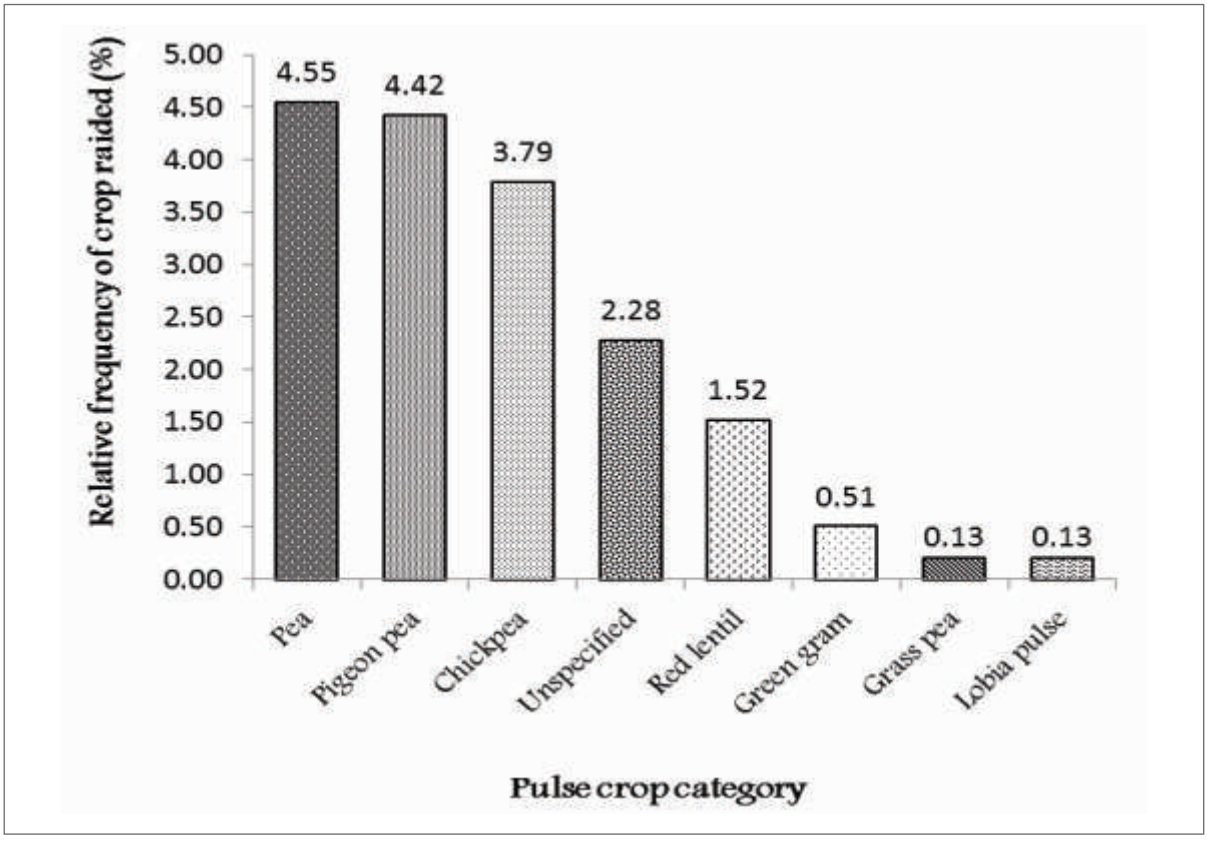


Figure 8.18 : *Relative frequency (%) of pulse crop categories raided by nilgai.*

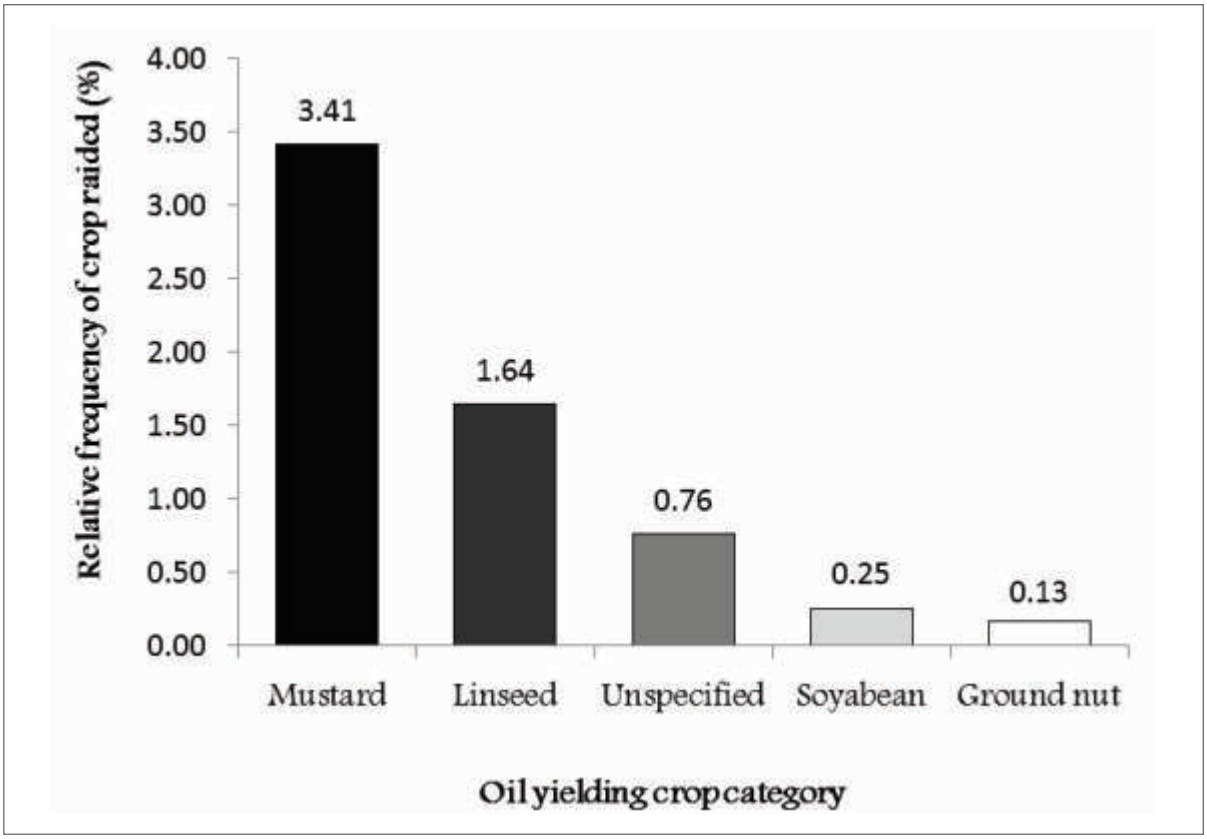


Figure 8.19 : *Relative frequency (%) of oil yielding crop categories raided by nilgai.*

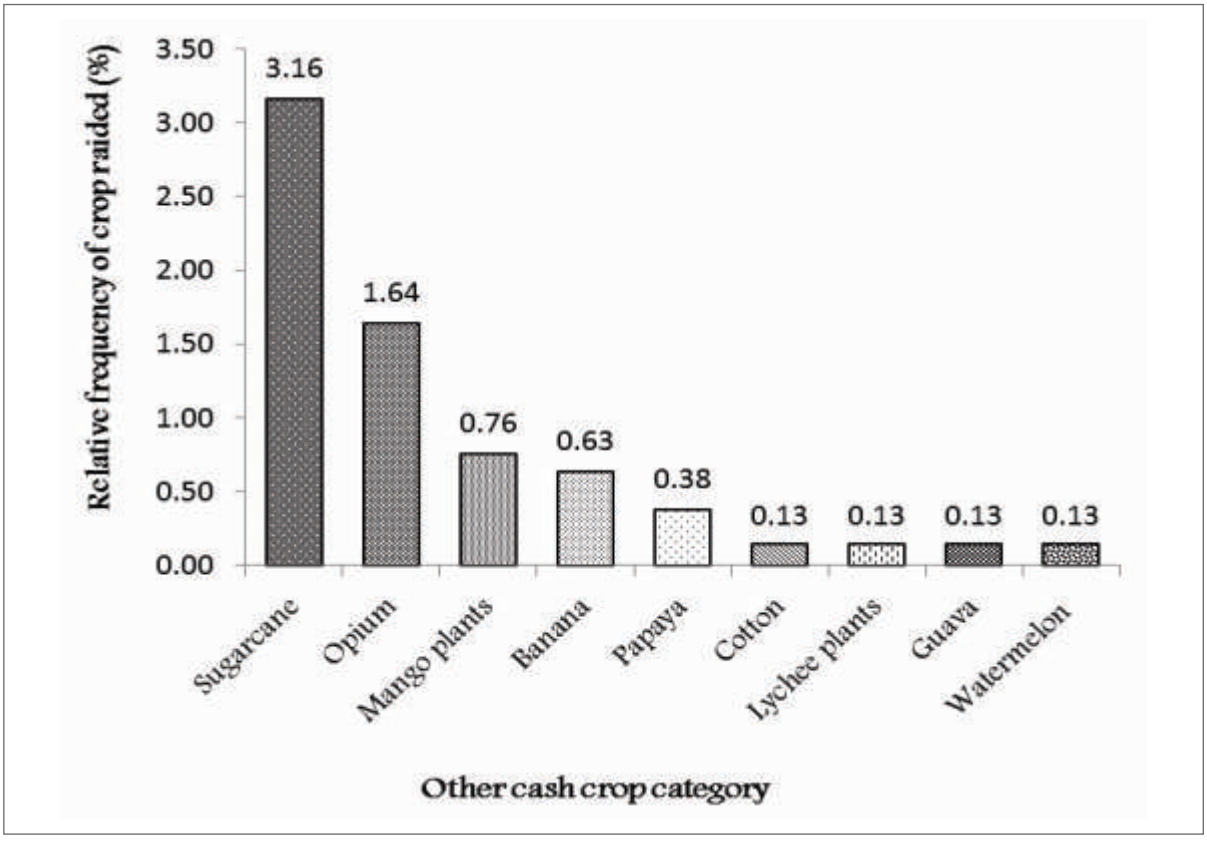


Figure 8.20 : *Relative frequency (%) of other cash crop categories raided by nilgai.*

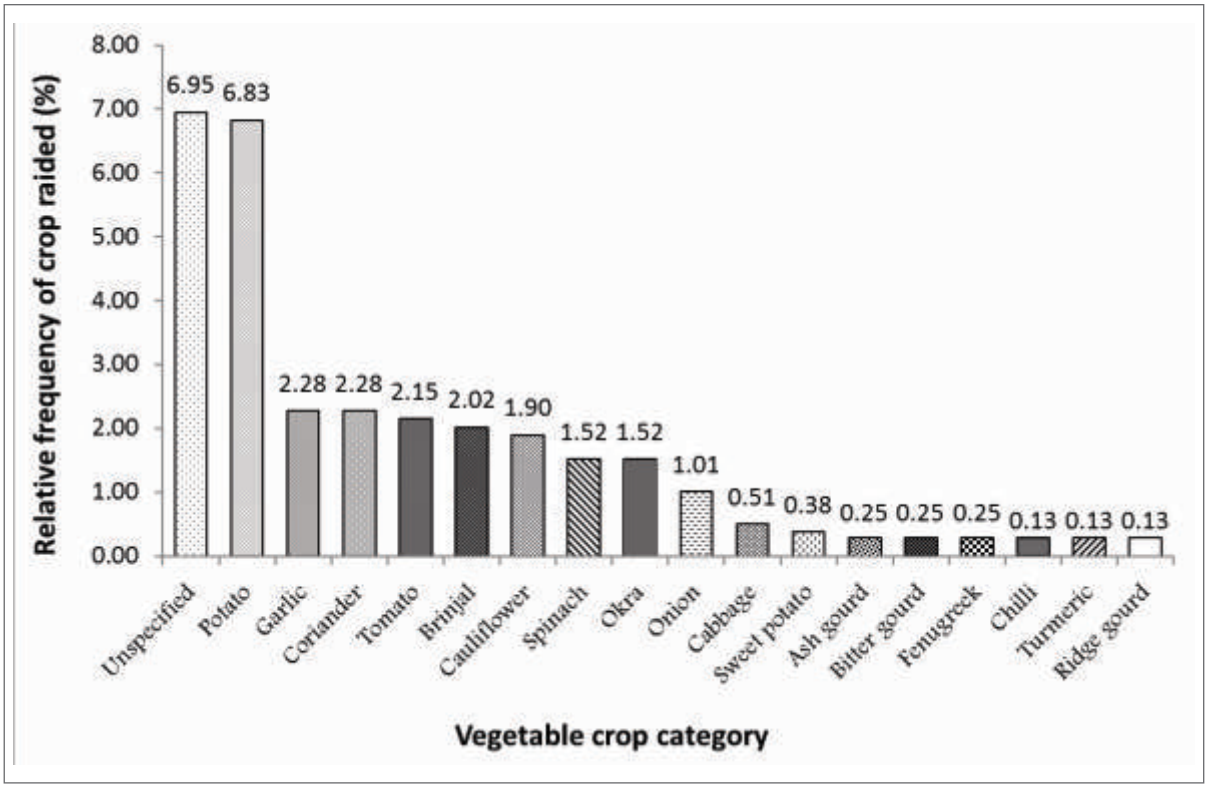


Figure 8.21 : *Relative frequency (%) of vegetable crop categories raided by nilgai.*

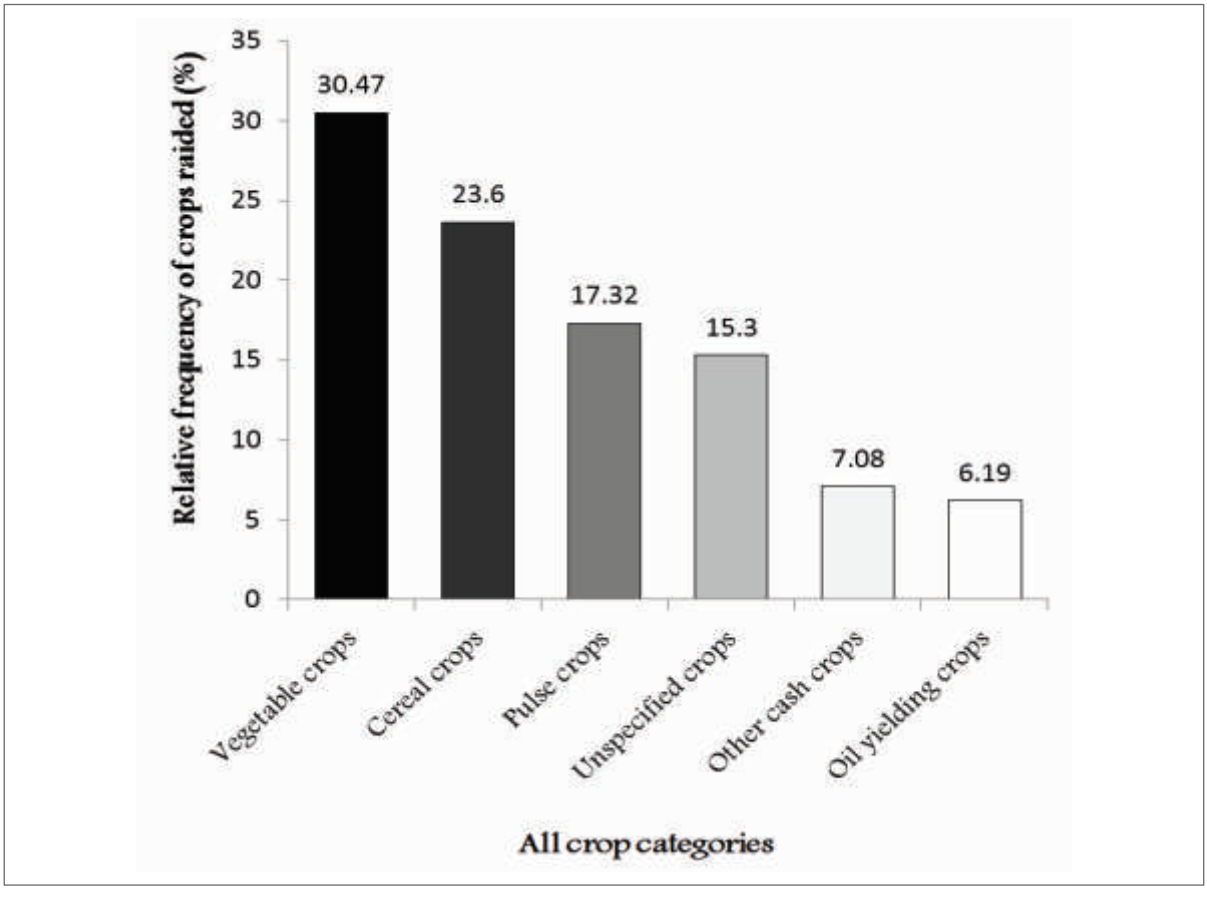


Figure 8.22 : *Relative frequency (%) of all crop categories raided by nilgai.*

SECTION IV

Laboratory Work

Chapter 9

NUTRITIONAL ECOLOGY OF FREE-RANGING RHESUS MACAQUES

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9.1 Dietary Analysis

Rhesus macaques inhabit diverse ecological habitats ranging from cold temperate to tropical latitudes. This diverse geographical distribution can be attributed to their adaptability in terms of behaviour and food habits. There is no doubt that rhesus macaques are thriving successfully as they are second only to human population in inhabiting such diverse landscape. Interestingly, rhesus macaque populations are concentrated around the areas of human inhabitation and causing various incidences of interaction. These macaque populations around human colonies are observed to be dependent on anthropogenic food in the form of garbage, orchards, provisioning by humans etc. When not mutual this interaction creates conflict as they cause huge damage to crops, orchards and gardens. Studying the habitat utilization by rhesus macaques will enable us to better understand their dependency on anthropogenic resources, the major cause of conflict. Rhesus macaques are considered as the most generalist amongst primates when it comes to foraging habits. It becomes important to thoroughly investigate how macaque populations thrive in areas with limited or no forest covers. Looking into nutritional ecology of these non-human primates will widen our knowledge in habitat utilization and nutrient intake balance achieved.

There are abiotic (climate, weather, latitude, landscape) and biotic (vegetation type, nutrient availability and composition) factors which influence the foraging habits of rhesus macaques (Machovsky- Capuska, Senior, Simpson, & Raubenheimer, 2016; Parker, 2003). Literature indicates that macaques achieve balance in their nutrition intake by complementary feeding, foraging on food resources imbalanced in nutrition with food having complementary imbalance (Felton, Felton, Wood et al., 2009; S. T. Guo et al., 2018; Johnson, Raubenheimer, Rothman, Clarke, & Swedell, 2013). In addition, the nutritional demand varies according to factors like age, stress, growth phase, reproductive status, infection, weather condition etc (Elder, 2009; Lambert & Rothman, 2015) thus, diverse diet offers the solution. Qualitative and quantitative assessment of rhesus macaque diet offers insight into the influence of human settlement in their foraging pattern, how increasing human activities are providing complementary foraging or disturbing their natural foraging.

Dietary flexibility is one of the major factors responsible for successful adaptation of an animal to its environment. The study area comprises of forest patches adjacent to human settlements, suburban areas, farmlands, orchards. The diversity in food resources is obvious as the macaques have access to provisioned food, garbage dumps and natural resources as well. In order to understand the resource utilization by macaques we are analysing collected faecal and plant samples for different parameters of dietary and nutritional assessment. The importance of forage quality to a sustainable and successful nutrition program cannot be overemphasized. Knowing forage nutrient content allows us targeting of forage resources to best meet animal needs, determines the need for additional supplements and evaluates which supplements best match the forage. Some routine and specialised chemical measures are used for evaluating dietary contents like crude protein (CP), Fat and carbohydrate content, Fiber content in terms of Neutral Acid Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) to best characterize forage quality. (Table no. 9.1)

Diet quality is often characterized by protein content since the assimilation of nitrogen, a key element of amino acids, determines animal growth. Protein availability may even have a greater limiting role than other food components, such as fats, especially during critical periods of the animal life cycle, such as the pregnancy, lactating, fetal development, and neonatal growth periods. NDF content is a good indicator of bulk fiber in the diet, whereas the ADF levels (negatively correlated) provide information on digestibility and thus energy intake. Lignin is the

Table 9.1 : Proximate principles for dietary assessment.

Essential Nutrients	Chemical Compounds	Analytical Procedures
Fatty acids, fat soluble vitamins	Lipids, pigments, sterols	Ether extract
Protein, amino acids	Nitrogen-containing compounds	Kjeldahl procedure (crude protein)
Inorganic minerals	Ash	Ashing (complete combustion)
Carbohydrates	Sugars, Starches	Nonstructual Carbohydrates
Glucose	Soluble fiber	Nonfiber Carbohydrates
	Hemicellulose, Cellulose	Neutral detergent fiber
Dietary Fiber	Lignin	Acid detergent fiber

major dietary insoluble fiber source; it may alter the rate and metabolism of other soluble fibers. Faecal Nitrogen concentration is having been found to be positively correlated with dietary protein (Holechek et al. 1982; Mould and Robbins 1981, Wofford et al.1985), intake (Arnold and Dudzinski 1963), dietary dry matter digestibility (Greenhalgh & Corbett, 1960; Lesile & Strakey 1985) and weight changes in mature game species (Gates and Hudson 1981). This positive relationship permits the use of chemical composition of faeces as an index of the quality of available forage.

9.1.1 Materials and methods

Study Area

The study site is a suburban area and adjoining forest cover, 4 different troops have been identified and each has a radio-collared adult female. Based on the data from collars, home ranges have been established for all the troops under study. For dietary analyses fresh faecal samples are collected and processed. Focal and behavioral sampling has allowed us to identify the plant species and their parts consumed by rhesus macaques. These identified samples are analyzed in laboratory for various nutritional components.

Dietary Assessment using faecal sample

Fecal samples were collected during transect walks and behavioral sampling to omit chances of irrelevant samples. Fresh samples were collected and stored in ziplock bags, labelled and transported to laboratory within 30 minutes of collection. For long term storage the samples were kept in -20^{o}C.

Sample Processing

Fresh faecal sample collected in zip lock with proper cataloguing.

Dry faecal sample in 60^{o}C in Hot air oven until sample were completely dried. (Samples are stored in -20^{o}C if not processing on same day).

Store the dry faecal sample in moisture free place.

Dried samples were weighed and processed for proximate principles analysis as per the mentioned protocols.

Dietary Assessment using forage plant sample

A total 63 plant species are identified to be foraged (as whole or parts) by Rhesus macaques of 4-study troops as per behavioural and direct observation from year 2019 to 2022. Plant or plant part(s) were collected and processed for proximate principles as described below (Table 9.2). Further these plant species are categorized based on seasonality, though some of the plans are foraged throughout the year, seasons are described based on rainfall and temperature.

Season	Month
Monsoon	July-Aug-Sept-Oct
Winter	Nov-Dec-Jan-Feb
Summer	Mar-Apr-May-June

Table 9.2 : List of dietary plant species for rhesus macaque in study area as observed during field focal and behavioral sampling.

S.No.	Common Name	Botanical Name	Parts being eaten	Season
01.	Doob grass	*Cynodon dactylon*	Leaf	Winter-Summer
02.	Ficus tree	*Ficus benjamina*	Leaf	Winter
03.	Plum	*Syzygium sp.*	Leaf-Fruit Flower	Winter-Summer Winter
04.	Lantana	*Lantana camara*	Leaf-Flower-Berries Leaf	Winter Summer
05.	Mango	*Mangifera indica*	Leaf-Twig Fruit	Winter-Summer Summer
06.	Neem	*Azadirachta indica*	Leaf-Fruit	Winter
07.	Sal tree	*Shorea robusta*	Leaf Flower-Fruit	Winter Summer
08.	Coralberry	*Ardisia sp.*	Leaf	Winter
09.	Hairy fig	*Ficus hispida*	Fruit Leaf	Winter Summer
10.		*Plantago sp.*	Seed	Winter
11.		*Flemingia sp.*	Leaf	Winter
12.	Cotton tree	*Bombax ceiba*	Leaf	Winter
13.	Roxburgh fig	*Ficus auriculata*	Fruit	Winter
14.	Flame of the forest	*Butea monosperma*	Leaf- Flower bud	Winter
15.	Dwarf copper leaf	*Alternanthera sessilis*	Leaf-Flower	Winter
16.	Glorybower	*Clerodendrum sp.*	Leaf	Winter
17.	Passion fruit	*Passiflora edulis*	Leaf	Winter-Summer
18.	Mulberry	*Morus sp.*	Leaf-Fruit-Flower Leaf	Winter Summer
19.	Wild Himalayan pear	*Pyrus pashia*	Leaf-Fruit Fruit	Winter Summer
20.	Indian pennywort	*Centella asiatica*	Leaf	
21.	Curry leaves	*Murraya koenigii*	Leaf	
22.	Karaunda	*Carissa opaca*	Leaf Fruit-Leaf	Summer Winter
23.	Kumkum	*Mallotus sp.*	Leaf	Summer
24.	Peepal tree	*Ficus religiosa*	Flower-Fruit Leaf	Summer Winter
25.	Common guava	*Psidium guajava*	Leaf-Fruit Leaf	Summer Winter

26.	Crown grass	*Paspalum sp.*	Seed	Summer
27.	Litchi	*Litchi chinensis*	Fruit	Summer
28.	Logwoods	*Xylosoma sp.*	Leaf	Summer
29.	Paper mulberry	*Broussonetia papyrifera*	Fruit Leaf	Summer Winter
30.	Mahaneem	*Melia azedarach*	Leaf	Monsoon
31.	Lime	*Citrus sp.*	Leaf	Monsoon
32.	Cleavers	*Galium sp.*	Leaf-Stalk	Winter
33.	White weed	*Ageratum sp.*	Leaf	Winter
34.	Hackberry tree	*Celtis tertrandra*	Leaf	Winter
35.	Multiflora Rose	*Rosa multiflora*	Leaf	Winter
36.	China Rose	*Hibiscus rosa sinensis*	Leaf-Flower	Winter
37.	White Jasmine	*Jasminum officinale*	Leaf-Flower	Winter
38.	Indian night shade	*Solanum indicum*	Fruit	Winter
39.	Black jack	*Bidens Pilosa*	Flower	Winter
40.	Congress grass	*Parthenium hysterophorus*	Leaf	Winter
41.	Amla fruit	*Phyllanthus emblica*	Fruit	Winter
42.	Chickweed	*Stellaria media*	Whole plant body	Winter
43.	Passion fruit	*Passiflora edulis*	Leaf	Winter
44.	Bamboo	*Bambusa vulgaris*	Leaf	Winter
45.	Turkey berry	*Solanum torvum*	Leaf- Fruit	Winter
46.	Ber	*Ziziphus sp.*	Leaf	Winter
47.	Creeping wood sorrel	*Oxalis corniculata*	Leaf	Winter
48.	Indian straw berry	*Duchesnea indica*	Leaf	Winter
49.	Marvel grass	*Dicanthium annulatum*	Leaf- Inflorescence	Winter
50.	Golden shower	*Cassia fistula*	Pod	Winter
51.	Water hemp	*Eupatorium sp.*	Leaf	Winter
52.	Cleavers	*Galium sp.*	Leaf	Winter
53.	Annual meadow grass	*Poa annua*	Inflorescence	Winter
54.	Burclover	*Medicago polymorpha*	Leaf	Winter
55.		*Setaria sp.*	Inflorescence	Monsoon
56.		*Carex sp.*	Leaf-Inflorescence	Monsoon
57.		*Cissampelos pareira*	Leaf-Flower	Summer-Monsoon
58.		*Lagerstroemia speciosa*	Leaf-Flower	Summer-Monsoon
59.	Kadam	*Anthocephalus sp.*	Leaf-Fruit	Summer-Monsoon
60.		*Bothriochloa sp.*	Leaf-Inflorescence	Summer-Monsoon- Winter
61.		*Eleusine indica*	Leaf-Inflorescence	Summer-Monsoon- Winter
62.	Nasturitium	*Tropaeolum sp.*	Flower	Winter
63.		*Colocasia sp.*	Pith-Root	Monsoon-Winter
64	Mushroom	*Termitomyces clypeatus*	Fruiting body	Monsoon
65	Mushroom	*Russula delica*	Fruiting body	Monsoon
66	Broom grass	*Thysanolaena sp.*	Pith	Monsoon

Sample processing (Plants)

Plant samples were collected, wet weight was taken and then dried at 60^{o}C in Hot air oven until sample were completely dried (for analysis plant samples were collected and processed same day).

Dried samples were powdered using grinder or mortar pestle, the resulting powder was then weighed.

If not processed on same day the powdered samples were stored in ziplock pouches with silica gel in moisture free place.

Dried samples were weighed and processed for proximate principles analysis as per the mentioned protocols.

Proximate principles analysis

A. Nitrogen

Reagents:

Magnesium oxide

Devarda's alloy

2% boric acid: Dissolve 20gm of H_2BO_3 power in warm distilled water and dilute to one litre.

Mixed indicator: Dissolve 70mg of methyl red and 100mg of bromocresol green in 100ml of ethyl alcohol. Add 10ml of this mixed indicator to each litre of 2% boric acid solution and adjust the pH 4.5 with dil. NaOH

0.01N Sulphuric Acid: prepare approximately 0.1N H_2SO_4 by adding 2.8ml of conc. H_2SO_4 to about 990ml of distilled water. Standardize it against 0.1N standard NaOH solution. Dilute 10 times this 0.1N H_2SO_4 to get strength of 0.01N.

Procedure

- Weigh 0.5gm of sample into digestion tube and moist with distilled water.
- Add 10ml of concentrated H_2SO_4 and 0.25g of catalyst and place the tube in digestion unit.
- Turn the heating equipment to about 400^{o}C and continue heating till the mixture is transparent blue and allow it to cool.
- Add 40% NaOH in digest till the colour change black and distill it.
- Collect the distillate (liberated ammonia) into 10ml of 2% boric acid solution.
- Titrate the distillate against 0.01N H_2SO_4 solution until pink colour starts appearing.
- Run a blank for each set of samples.

Calculation

$$N^{0}/_{0} = \frac{(S-B)x\ Nx1.407}{sample\ weight\ (g)}$$

Where,

S= volume of acid used against sample
B= volume of acid used against blank
N= normality of acid
Crude Protein (CP)% = %N x 6.25

B. Fiber

Neutral Detergent Fiber (NDF)

Apparatus: Spout less beakers(100ml), round bottom condenser, hot plate, sintered crucibles (G1 grade), vaccum pump, muffle furnace or fiber analyzer

Reagents

Neutral Detergent Solution (NDS): add 30g of sodium lauryl sulphate; 18.61 g Ethyleneaminetetraacetic Disodium Salt (EDTA); 6.81g sodium tetraborate dehydrate; 4.56g sodium phosphate dibasic(anhydrous) and 10 ml of triethylene glycol in 1000ml distilled water. Stir mix in heat to facilitate solubility. Check pH range to 6.9 to 7.1

- **Sodium sulfate:** Na_2SO_4 anhydrous
- **Acetone:** Extra pure or AR grade or use grade that is free from color and leaves no residue upon evaporation.
- Decahydronapthalene
- N-octanol

Procedure

- Weigh 0.5g of air dried sample (W2) into spout less beaker
- Add 100 ml of detergent solution (NDS), 0.5 g of sodium sulfate and 2ml of Decahydronapthlene
- Reflux for 60 minute after boiling.
- Filter through a pre weighed crucible, rinse the sample with hot water, filter the liquid and repeat the washing process 3-5 times.
- Wash twice with acetone and dry by suction
- Allow acetone to evaporate and after that complete drying process in oven at 105^{o}C at least for 8 hours and weigh (W3)
- Ash entire sample for 2 hours at 550^{0}C, cool in desiccator and weigh (W4)

Calculation

$$NDF\% = \frac{W3-W1}{W2} x\ 100$$

$$NDF\ OM\ (DM\ Basis)\ \% = \frac{W4-W1}{W2} x\ 100$$

Where,

W1= empty crucible weight

W2= sample weight

W3 = weight after extraction process

W4= Weight of organic matter (OM)- loss of weight on ignition

Acid Detergent Fiber (ADF)

Apparatus: same as used in NDF estimation

Reagents

- Acid Detergent Solution (ADS): Add 20g cetyl trimethylammonium bromide (CTAB) to 1-liter 1N H_2SO_4
- Acetone: extra pure or AR grade or use grade that is free from colour and leaves no residue upon evaporation
- Decahydronapthlene
- N-hexane or n-octanol

Procedure

- Weigh empty crucible (W1) record weight and tare balance
- Weigh 0.5g of air-dried sample (W2) ground to pass through a 1mm screen (2mm screen when using a cyclone mill) in spout-less beaker
- Add 100ml of acid detergent solution (ADS) and 2 ml of decahydronapthalene
- Reflux for 60minutes after boiling
- Filter through a pre weighed crucible, rinse the sample with hot water. Filter liquid and repeat the washing process 3-5 times
- Wash twice with acetone and dry by suction.
- Remove hexane by suction if crucible contains some acetone
- Ash entire sample for 2 hours at 550^{0}C, cool in desiccator and weigh (W4)
- If doing ADL then not put in muffle furnace

Calculation

$$\text{ADF (as-is-basis)} = \frac{W3-W1}{W2} \; x\; 100$$

$$\text{ADF (DM basis)} = \frac{W3-W1}{W2 \times DM} \; x100$$

$$\text{ADF OM (DM basis)} = \frac{W4-W1}{W2 \times DM} \; x100$$

Where,

W1= empty crucible weight

W2= sample weight

W3= weight after extraction process

W4= weight of organic matter (OM) - loss of weight on ignition

Acid Detergent Lignin (ADL)

Estimation of ADL requires preparation of ADF first

Apparatus: Same as required for ADF-NDF

Reagents

Sulphuric acid (72%) w/w: take 1200ml distilled water in volumetric flask of 2000 ml and 800 ml of concentrated H_2SO_4

Procedure

- After estimation of ADF, fill the crucible with 70% H_2SO_4, stir with glass rod to smooth the paste and break the lumps.
- Refill with sulfuric acid and stir at hourly intervals as acid drains way. Give a total three addition.
- After three hours, filter off as much acid as possible. Wash content with hot water 3-5 times
- Dry in oven at 105ºC for at least 8 hours and weigh (W5)
- Ash entire sample for 2 hours at 550ºC, cool in desiccator and weigh (W6)

Calculation

$$\text{ADL (ADL\%)} = \frac{W5-W6}{W2} x100$$

Where,

W5= weight after lignin extraction

W2 = sample weight at ADF initiation

W6= weight after ash (crucible + ash)

Results

9.1.2 Proximate principles analysis (Dietary fiber and Nitrogen) from fecal samples

Individual sample values are plotted where red line indicates the mean value, the spread of values can be attributed to lack of seasonality and small sample size (Table 9.3). The samples collected are random i.e. irrespective of sex, age, reproductive status, physiological condition. For an appropriate season wise comparison higher number of samples need to be processed and analysed. Figure 9.1, represents the estimated faecal nitrogen percentage for each troop, troop B has significantly higher Nitrogen content in comparison to troop A($P<0.05$); pairwise

Table 9.3 : Summarized values of assessed dietary components from faecal samples.

Troop	% Nitrogen Mean ± S.E.	% NDF Mean ± S.E.	% ADF Mean ± S.E.	% Lignin Mean ± S.E.
A	2.794 ±0.1434	51.88 ±5.78	62.65 ±4.618	41.78 ±8.061
B	3.456 ±0.1936	65.24 ±1.9	50.31 ±3.247	31.56 ±10.54
C	3.235 ±0.1673	52.80 ±6.16	60.51 ±2.39	41.07 ±8.638
D	2.962 ±0.1563	49.93 ±4.8	61.48 ±2.64	33.52 ±4.332

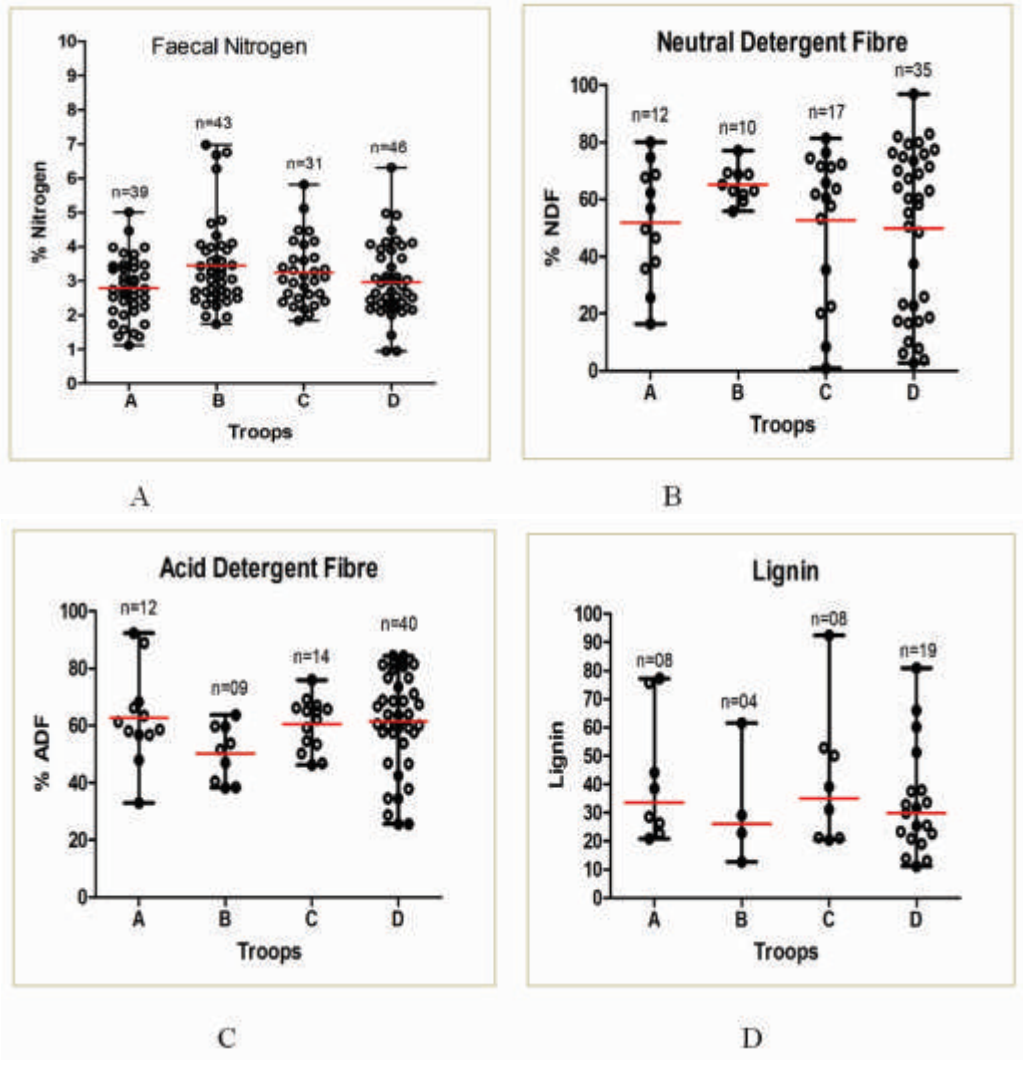

Figure 9.1 : *Assessment of Dietary components from Rhesus macaque faecal samples.*

comparison amongst rest of the troops showed no significant difference. Figure 9.1 B, C and D represent the fiber content determined in faecal samples of four troops, no significant difference was observed. The data was subjected to one-way ANOVA with Bonferrroni post-hoc test to check for significant differences.

- Proximate principles analysis from plant samples

Proximate principles were assessed for collected plant and/or parts samples (Fig. 9.2 & 9.3); as per observation the moisture content of different plant parts leaves, flowers, fruits and grass was similar. Fibre content was higher in leaves and grasses. Nitrogen (expressed as protein %) content was higher in grasses as compared to other plant parts. Nitrogen estimation is correlated to protein content but the availability of the estimated nitrogen in organic and inorganic form needs to be determined by simultaneous analysis of faecal nitrogen content.

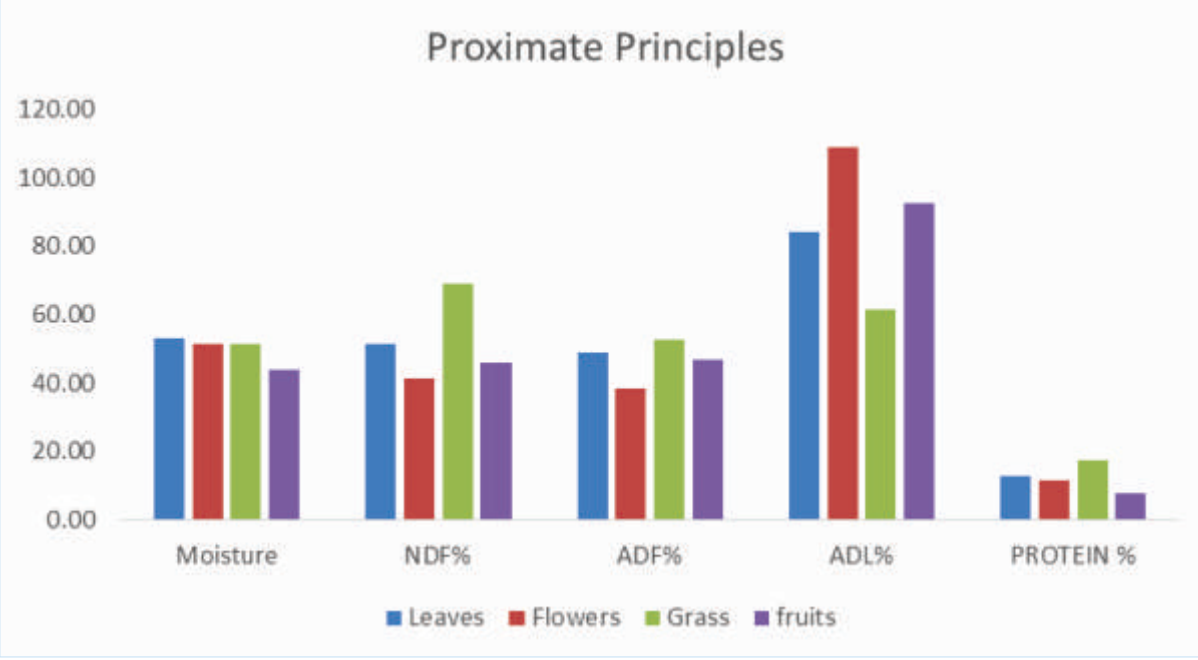

Figure 9.2 : *Proximate principles analysis from identified and collected macaque dietary plant samples.*

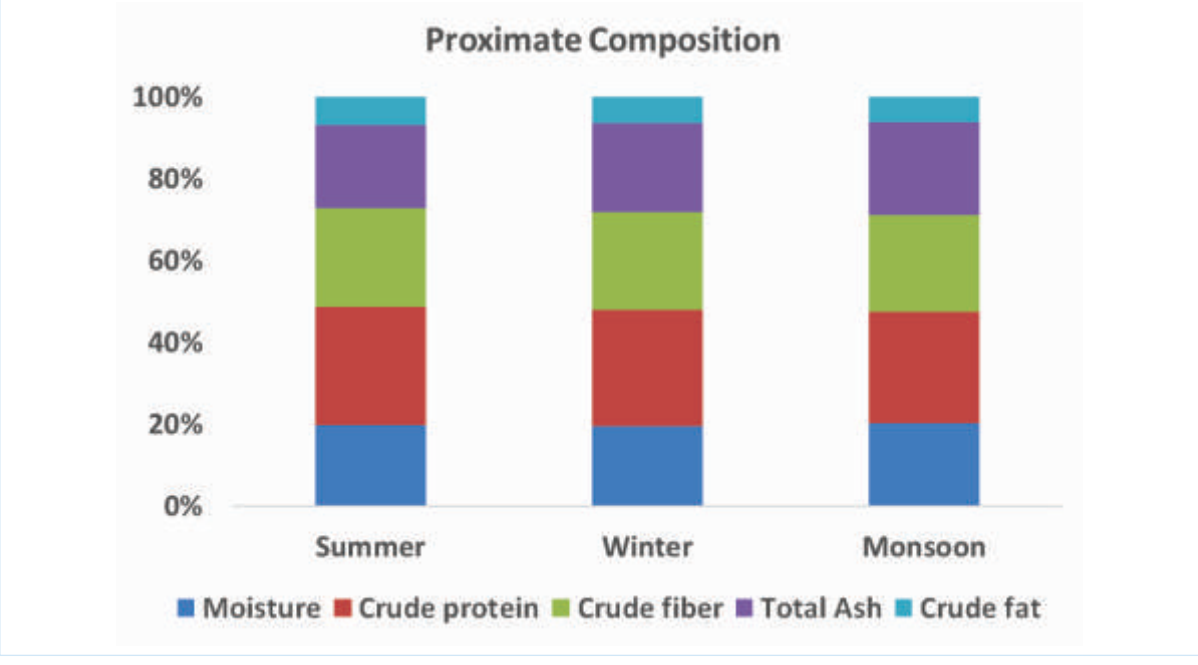

Figure 9.3 : *Proximate composition (in percentage) of Dietary plant sample*

Table 9.4 : Proximate composition (in percentage) of Dietary plant samples categorized seasonwise.

Season	Moisture %	Crude protein %	Crude fiber %	Total Ash %	Crude fat %
Summer	10.28	14.97	12.45	10.56	3.56
Winter	10.59	15.51	12.97	11.86	3.45
Monsoon	10.30	13.98	12.05	11.52	3.23

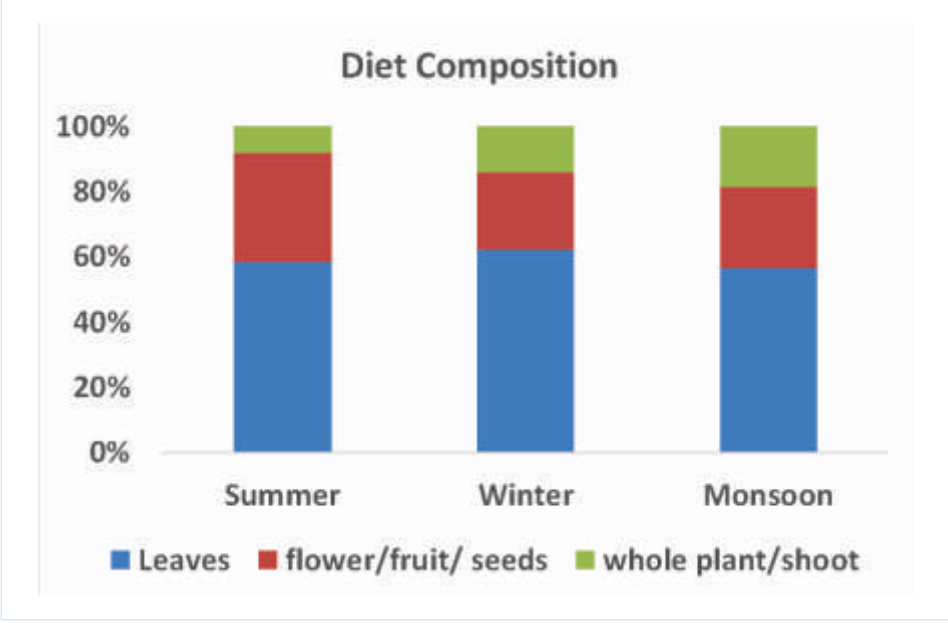

Figure 9.4 : *Diet Composition of different plant parts consumed by Rhesus macaque seasonally.*

Table 9.5 : Diet Composition Percentage of Dietary pattern in Rhesus macaque.

Season	Leaves	Flower/fruit/ seeds	Whole plant/shoot
Summer	14.00	8.00	2.00
Winter	13.00	5.00	3.00
Monsoon	9.00	4.00	3.00

As observed during behavioral sampling (foraging behavior), rhesus macaques fed on various plant species growing in the study area. Some plants were consumed as whole whereas for others different plant parts like leaves, flowers, fruits, seeds, shoots were consumed. Their diet majorly comprises of plant leaves across the seasons (as depicted in the graph above). In natural ecosystems seasons regulate availability of different plant parts, immature leaves, flowers, fruits, seeds thereby inducing periods of food abundance (Sengupta and Radhakrishna 2016). Despite seasonality leaves are good source of fiber, protein and minerals; are available throughout the year and in different stages (young and old) making them a preferred source of food.

Chapter 10

ANIMAL HEALTH STUDIES – AN IMPORTANT ONE HEALTH CONCERN AT THE INTERFACE

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Rhesus macaque

10.1 Anti-microbial Resistance Studies

Isolation and characterisation of antimicrobial resistant bacteria

Rhesus macaque, one of the most widely distributed non-human primate (NHP), can act as a host to several zoonotic pathogens. Epidemiologists and infectious disease ecologists consider primates to be potential disseminators or super-spreaders of parasites into human populations, given their long-shared evolutionary histories (Hasegawa et al. 1985). With the growing number of studies on primate infectious disease ecology, it has been observed that among the five major taxonomic groups of endoparasites (i.e., viruses, bacteria, fungi, protozoa, and helminths), not all have been intensively explored. There is well- documented evidence in primate populations for the prevalence of three groups, i.e., viruses (Jones-Engel et al. 2001, 2007; Engel et al. 2008), protozoa (Ekanayake et al. 2006; Lee et al. 2011), and helminths (Gotoh et al. 2001; Lane et al. 2011) but the prevalence of enteric bacterial parasites remain less assessed (Nunn and Altizer 2006). Bacterial endoparasites are commonly acquired and shed by humans (Nunn and Altizer 2006). Among the spectrum of pathogen harboured by

macaques, enterobacterial pathogens including *Shigella* spp., *Salmonella* spp., and *Escherichia coli* are the most commonly distributed pathogens in non-human primates and are often transmitted to human and domestic animals by water sources and domestic environment contaminated with faeces and vice versa as bacterial endoparasites are also commonly acquired and shed by humans (Wolfe LD. 2002, Nunn and Altizer 2006, N.D. Wolfe et al., 2007, B. A. Beisner et al.,2016, M. R. McLennan et al., 2018). Urban rhesus macaques inhabiting areas dominated by human settlement therefore become more susceptible to acquiring these bacteria. Their ability to exchange pathogens with human and livestock has been reported (Wolfe et al.,2007).

The gut bacteria of wild animals are influenced by the proximity to human habitation because of densely populated microbial habitats in which antibiotics select for resistance. The implications of the occurrence of antimicrobial resistance in wildlife are that wildlife serves as an environmental reservoir and a melting pot of resistance. This study will tell us the role of macaques in enterobacterial zoonoses by examining the microflora obtained from freshly collected faeces and their role in maintenance and transmission antimicrobial resistance which is a global emerging health concern. Also, changes in the composition of the gut microbial community are known to lead to changes in its function, which can alter host nutrition, health and behavior. Environmental factors such as diet or social contact are largely responsible for determining the composition of the gut microbial community (Amato,2013). Therefore, studying the gut microbiota reflects upon the host's nutrition and health status and hence will give an insight on this as well. The procedures followed for the same is described below.

Methodology

Sample Collection & Processing: Fresh faecal samples from free ranging macaques/rectal swabs from captured individuals were collected in BHI broth from study troops in and around Chandrabani area and processed on the same day.

Sampling was done once a week and processed for the rest of the week till single colonies of pure cultures were stored in glycerol stock.

Media used for isolation of pure cultures: Brain Heart Infusion Broth (BHI), Rappaport Vassiliadis Broth for enrichment of samples and MacConkey Agar (MLA), Eosin Methylene Blue Agar (EMB), Salmonella Shigella Agar (SSA), Hektoen Enteric Agar (HEA) and Brilliant Green Agar (BGA) for isolation of bacteria from the enriched samples.

Methodology for culturing: A sterile inoculation loop was inserted into the feacal sample and enriched in BHI broth and Rappaport Vassiliadis Broth separately followed by 18-24 hours of incubation at 37^{o}C. Subsequently BHI enriched cultures were streaked on MLA and Rappaport enriched cultures on SSA and incubated for 18-24 hours at 37^{o}C. Further separate colonies were isolated from the mixed culture on MLA and grown on EMB, HEA, BGA and incubated for 18-24 hours at 37^{o}C. Single colonies from pure cultures hence isolated were stored in glycerol (50%) for further molecular studies.

Antibiotic Sensitivity Test: The antibiotic susceptibility profile of the Gram-negative isolates was determined using the standard Kirby-Bauer disk diffusion method.

Methodology for Antibiotic Sensitivity Test: Bacterial culture suspension was spread on Muller-Hinton agar plates using L spreader and a maximum of 6 antibiotic disks were used on a single petri plate and incubated at 37^{o}C for 18-24 hours; then, the inhibition zone diameters around the antibiotic disks were measured. The results were expressed as susceptible or resistant according to the criteria recommended by the CLSI. As per plan, the phenotypic study will be followed by molecular confirmation of resistant genes present in these isolates based on the phenotypic study results.

Double disk synergy test: Isolates which were resistant to more than three classes of antibiotics were tested for the confirmation of ESBL. Test isolates were spread on Mueller Hinton Agar plates following the same method used for AST. Amoxicillin, ceftazidime and cefotaxime were chosen for DDST with their respective combination drugs amoxicillin/ clavulanic acid (20/10mcg), ceftazidime/clavulanic acid (30/10mcg), cefotaxime/clavulanic acid (30/10mcg). Each antibiotic was placed with its combination drug in each plate incubated at 37^{o}C for 18-24h as per the CLSI guidelines.

Detection of virulence genes: Samples were tested for the detection of Shiga-like toxins stx1, stx2, hlyA and eaeA.

Results

Altogether 110 samples from the 4 study troops have been processed so far, out of which all 94 samples are suspected to be *E.coli* positive based on their morphology on the EMB agar plates. 16 samples are suspected to be *Salmonella* sp. positive based on their morphology on the SSA plates and 20 samples have shown colony morphology similar to that of non-lactose fermenting Enterobacteriaceae on EMB agar which would be confirmed after genetic analysis.

The antibiotic susceptibility profile (Phenotypic characterization of AMR) of the Gram-negative isolates was determined using the standard Kirby-Bauer disk diffusion method on Mueller Hinton Agar (MHA). For standardization of the protocol, 3 suspected *E.coli* isolates were tested with Amikacin (30mcg), Erythromycin (15mcg), Streptomycin (10mcg), and Tetracycline (30mcg). All samples were sensitive to Amikacin, Streptomycin and tetracycline except for one which showed intermediate resistance to tetracycline. All samples were also resistant to Erythromycin owing to intrinsic resistance. These isolates shall be further subjected to phenotypic antibiotic sensitivity assay. Additionally, for Genotypic characterization of AMR, DNA was extracted from isolates and subjected to multiplex PCR with primers that correspond to conserved regions of beta lactam encoding genes- blaTEM, blaSHV, blaCTX-M and blaIMP. 62 isolates were phenotypically resistant to more than three classes of antibiotics were subjected to multiplex PCR with specific primers. Beta-lactam gene blaSHV was the most detected gene followed by blaCTX-M and blaTEM.

Shiga toxin stx1gene was detected in 1sample and eaeA gene was detected in 5 samples.

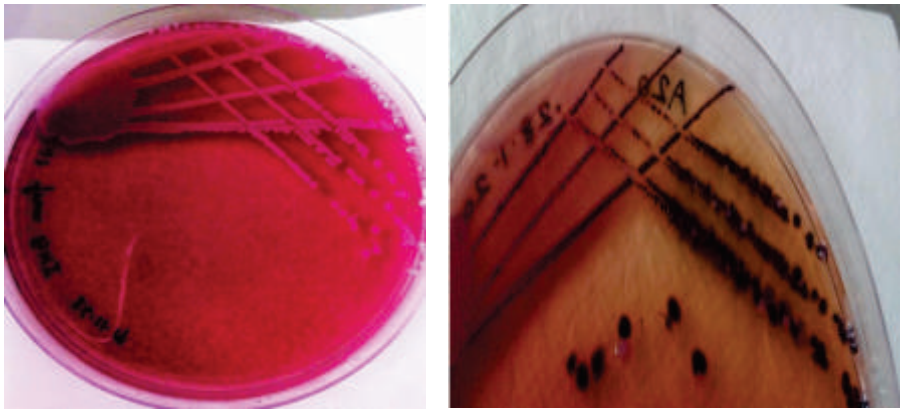


Figure 10.1 : *Colonies of lactose-fermenting bacteria on MLA (L); Bright orange colonies on HEA metallic shine indicating growth of E.coli / Klebsiella sp. (R).*

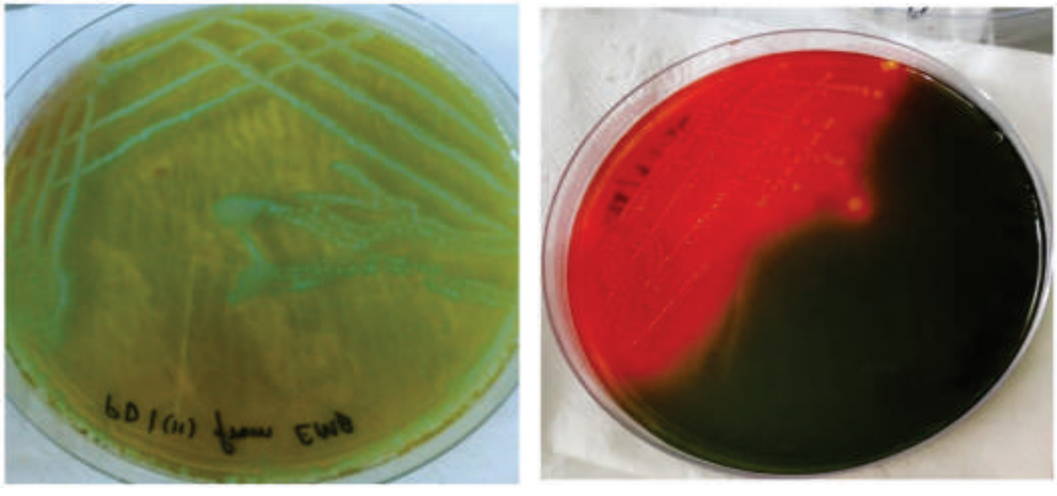


Figure 10.2 : *Yellowish green colonies on BGA black centres indicating growth of E. coli (L); Colourless colonies with black centres on SSA indicating growth of Salmonella spp. (R).*

Pharmacy survey

We have surveyed 22 pharmacies located near the study area (16 sq.km.) to know the most sold antibiotics to correlate with phenotypically resistant isolates. Our survey showed 10 antibiotics, belonging to 6 major antibiotic classes, to be the most prominently traded medications in this category for human ailments at the local pharmacies which are located near our study area. This will allow us to investigate if higher sales of a particular antibiotic or class showed any correlation to their resistance in microbial cultures from *Macaca mulatta* faecal samples collected from the study area.

10.2 Gastrointestinal Parasite Studies

10.2.1 Gastrointestinal Parasite Studies across areas of varied human use intensity (year 2018-19)

The realm of parasite ecology focuses on the interactions between hosts and parasites as a system. Parasitism is a natural phenomenon, where host-parasite ecosystems have evolved

simultaneously. It is viewed as one of the limiting factors for wild populations, along with others like predation and competition (Caughley and Krebs, 1983). Several genera of endoparasites, including both gastro-intestinal and haemoparasites are known to infect all major clades of living non-human primates (Nunn et al., 2003). Humans and anthropoid primates broadly share similar physiological and genetic characteristics. This makes them susceptible to many parasites which can potentially cross interspecific boundaries of transmission (Mücke 2011). A detailed parasitic study was thus carried out from January – April, 2019, with the aim to understand the host - parasite dynamics in a population of free-ranging commensal Rhesus macaques, across areas of different intensities of human use in Uttarakhand (Figure 10.3).

Methods & materials

Faecal sample collection: Faecal samples were collected from the study troops A, B, C, D (high human use) and E, and F (Low human use). A total of 67 faecal samples were collected from Chandrabani and 77 were collected from Rajaji and Chidiyapur combined.

Fresh faecal samples of individuals were collected from the cage/during capture/ during behavioral sampling in containers with 10% formalin and stored at 4^{o}C until lab analysis was done. These samples were marked with the approximate age and sex of the animal.

Prevalence of Gastrointestinal parasites in free-ranging Rhesus macaques

(i) Qualitative analysis

For concentrating the parasite eggs in the faecal sample, flotation and sedimentation method

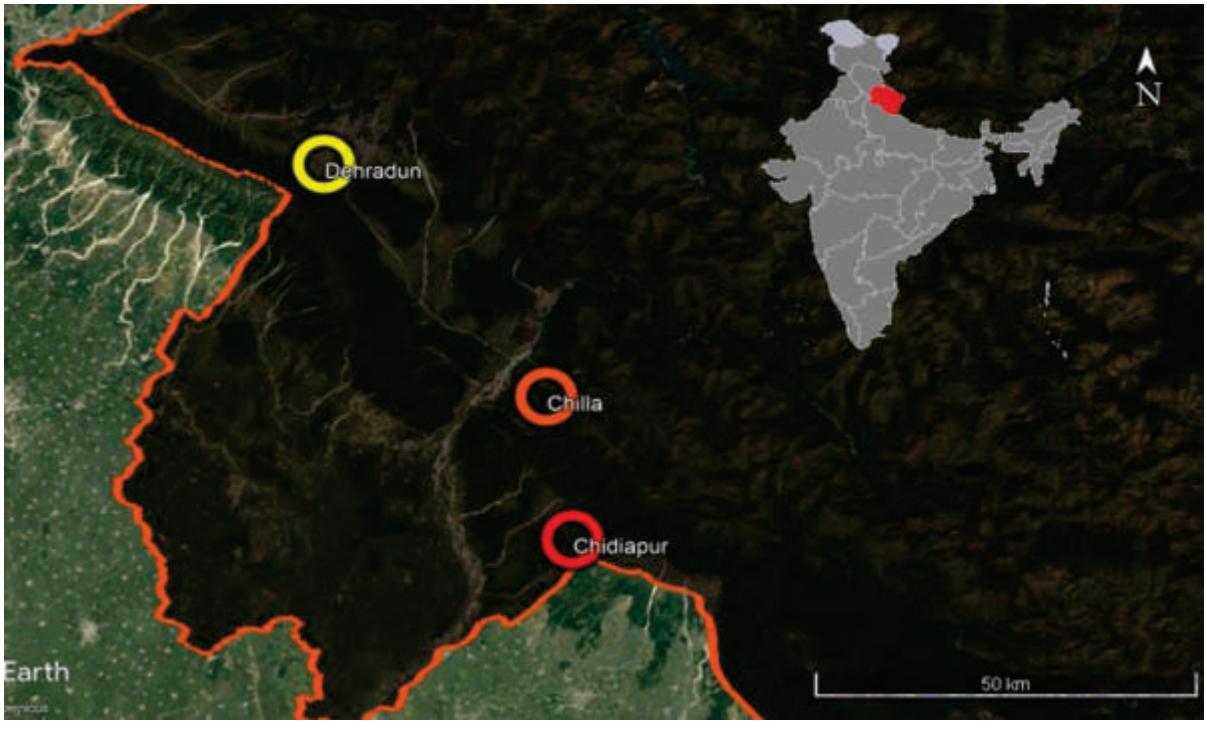


Figure 10.3 : *Study areas (circled). The Yellow circle depicts an area of high human use (Chandrabani) and the red (Chidiapur) and orange (Rajaji National Park) circles depict the two study sites of an area of low human use.*

was carried out.

Briefly, using gloved hands, the weighed-out portion of the faecal sample was put in approximately 5 ml of NaCl solution and mixed well with a mortar and pestle. This well-mixed slurry was then sieved through a tea-strainer and the fibrous contents of the faecal sample were discarded. The filtrate was transferred to a 15 ml centrifuge tube and filled to the brim to create a positive meniscus. A coverslip was then placed on this and the set-up was left to stand for 15-20 mins. The coverslip was gently removed from the top of the centrifuge in such a way that the drop of the faecal solution was hanging on the coverslip. This was then placed on a glass slide and observed under a microscope under 10x and 40x magnification. Every slide was scanned until all the fields of the coverslip were covered which took upto 20- 30 minutes per sample. Parasite eggs were photographed for identification.

Detection of Eggs: The sedimentation technique is useful to concentrate trematode eggs which are heavier than nematode eggs. As individual macaque faecal samples are comparatively lesser in quantity than that of ungulate/ domesticated livestock, the samples had to be used judiciously. Thus, sedimentation was carried out with the same sample as the one processed for flotation. This technique was developed and routinely recommended for less quantity of faecal samples by the Veterinary Parasitology Department, Indian Veterinary Research Institute (IVRI), Izatnagar (personal communication). After flotation is completed, the heavier contents of the faecal sample settle down at the bottom of the test tube. The flotation solution is decanted carefully and refilled with salt solution and allowed to settle again. This is done until a clear supernatant is obtained and this clear supernatant is carefully discarded. The remaining sediment at the bottom of the test tube is then smeared on a glass slide and covered with a coverslip to examine for any trematode eggs.

Presence of parasite larvae and eggs were recorded and photographs were taken for every unique egg or individual found within a slide. Size, shape, colour of ova and helminth eggs were recorded in detail and used for further identification of the parasitic taxon. Identification keys were used from published literature for the identification (Soulsby, 1968).

(ii) Quantitative analysis

In order to determine the number of eggs present per gram of faeces (eggs per gram/ EPG), the McMaster counting technique is used most commonly. A weighed-out portion of 1 gm of the faecal sample was thoroughly mixed with 14 ml of the flotation solution, thoroughly mixed and strained. This strained solution was then taken with a dropper and loaded on to the 2 chambers of a McMaster slide. This slide was allowed to rest for 5 minutes until the eggs floated near the surface of the slide for easier detection, and was then observed under 10x magnification lens. The eggs within the counting chamber were counted and the total of both the chambers was added. This sum of the eggs of the 2 chambers was then multiplied by 50 to obtain the egg per gram count of the faecal sample.

Results

Prevalence of gastrointestinal parasites in free-ranging Rhesus macaques

(i) Qualitative Analysis/ Identification of Parasites

The identification of parasites is done by observing the morphological differences in the parasitic eggs as tabulated below. Morphological identification is helpful for discerning the parasite up to family level especially within the families of Strongyles and Strongylids since the egg morphology of several species within them is very similar. The eggs for these families are thin shelled. All three families are similar with respect to egg size (Soulsby, 1968).

Table 10.1 : Characteristic Feature of parasites found in Rhesus macaque faecal sample.

Family	Characteristic Feature
Strongyles	This family can be identified by the presence of a morula within the egg (Figure 10.4 A).
Strongyloides	The identification pointer to this family is the presence of a L2 stage of larvae within the egg (Figure 10.4 B).
Trichurids	Trichuris eggs are characterized by the presence of two polar plugs at the ends of the egg (Figure 10.4 C).

(A) (B) (C)

Figure 10.4 : *Identification of parasites using microscopy*
(A) Strongyle spp. (B) Strongyloides spp. (C) Trichuris spp.

(ii) Quantitative Analysis

a) Percentage prevalence of parasites in the two study areas

A higher percentage of mixed infections with *Strongyles*, *Strongyloides* and *Trichuris* was found in Chandrabani (high human use) in comparison with Rajaji-Chidiyapur (low human use). All three parasite genera were detected individually in the single parasite infections.

Table 10.2 : Single and mixed infection percentage among Rhesus macaques in the study area.

Study area	Samples with single parasite genera in same individual	Samples with mixed infection (>1 parasite genera in same individual)	Samples negative for parasites
Chandrabani	16.66% (11)	12.12% (8)	71.21% (47)
Rajaji + Chidiyapur	3.89% (3)	5.19% (4	90.90% (70)

The number of individuals infected amongst a population was higher in Chandrabani (high human use) than in Rajaji (low human use). For Strongyle and Strongyloides there was a significant difference in the percentage prevalence of infected individuals in the two areas. However, for Trichuris, the difference in prevalence between the two areas was not significant.

Percentage prevalence of various parasite species in the two study areas

The EPG count shows that the mean EPG in area of high human presence is significantly higher 264 than that in area of low human presence (Mann-Whitney U test, p-value = 0.04), thus 265 Chandrabani area has a higher parasite load than Rajaji-Chidiyapur area (Figure 10.5).

10.2.2 Gastrointestinal parasite studies in Rhesus macaque, Chandrabani area (year 2021-22)

Rhesus macaque (*Macaca mulatta*) is a species of old world monkey that is most widely distributed in south Asia and can easily adopt to a broad range of habitats (Timmins et al., 2008). Rhesus macaque have tendency to move towards the areas near human dwelling in pursuit of food (Chapman & Perez, 2001; Chapman et al., 2005; Ciani, 1986). Close contact between Rhesus macaque and humans as a result of dependency on anthropogenic food sources increases the chance of zoonotic parasite spill over (Daszak et al.,2001). Parasite

Table 10.3 : The number of infected individuals in a population (prevalence).

Parasite	Chandrabani (% prevalence)	Chidiyapur + Rajaji (% prevalence)	p-value (Difference of prevalence between areas)
Strongyles	21.21%	6.49%	0.019
Strongyloides	21.21%	7.79%	0.038
Trichuris	7.57%	1.29%	0.147
All three	21.21%	7.79%	0.038

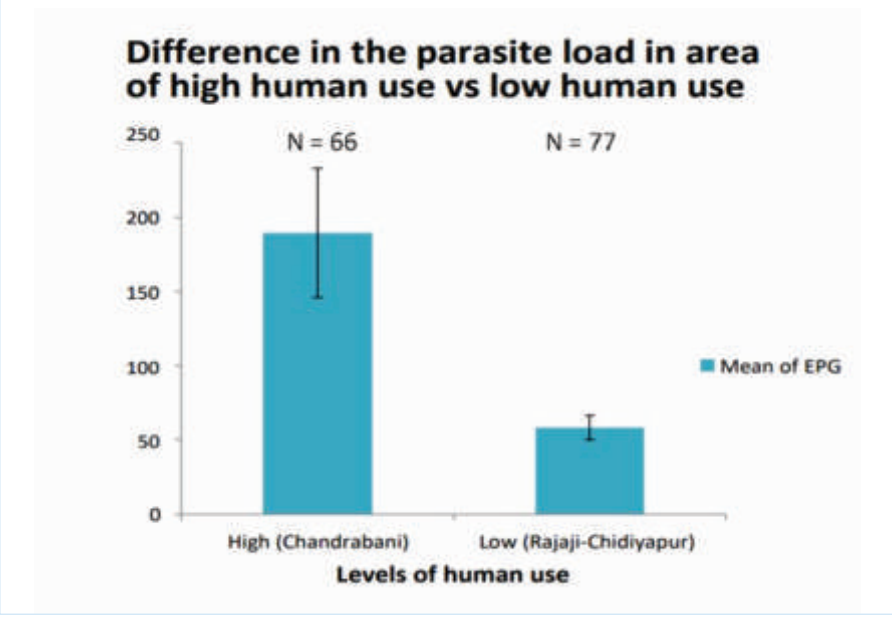


Figure 10.5 : *Comparative graph of parasite load across areas of high vs low human use.*

infection dynamics within macaque groups depend on host factors including age, sex, body size, dominancy, immunity and general health status, and sociological factor including group size, social behaviour, population density, and habitat factor. Continuing the above-mentioned study, we proceeded to identify and investigate the prevalence of Gastrointestinal parasites in free-ranging Rhesus macaques in Chandrabani, Dehradun.

We initiated baseline studies with the following objectives

(1) To investigate the prevalence and abundance of gastrointestinal parasites in Rhesus macaques

(2) To determine the correlation between haemato-biochemical parameters and parasite prevalence on the health of Rhesus macaques in study area.

- **Prevalence of Gastrointestinal parasites in free-ranging Rhesus macaque**

Monkeys are particularly susceptible to parasitic infections because they live in cohesive groups characterized by frequent social interactions (Stoner, 1996) and specific feeding and drinking behavior (Pokhrel & Maharjan, 2014), which facilitate parasite transmission between individuals (Adhikari and Dhakal, 2018). The objective our study is to understand the prevalence of gastro-intestinal parasites in four study troops of Rhesus Macaque in chandrabani area, Dehradun. Based on observation of Shariful et al. 2021, study conducted in Bangladesh in free ranging Rhesus macaques reported significantly higher prevalence of Gastrointestinal parasites in macaques inhabiting rural areas, where macaques frequently interact with humans and other domestic animals.

Interactions between humans and free ranging macaques are common in Chandrabani area these type of interaction may increase the risk of bidirectional disease transmission. Rhesus macaque troops cohabiting human settlement use water bodies and garbage dumps which facilitates the contact with contaminated resources increasing the opportunities for cross-species transmission of Gastrointestinal parasites.

- **Haemato-Biochemical studies and Prevalence of parasite in free-ranging Rhesus macaques**

The objective of the study is to know the prevalence of parasitic species of the Rhesus macaque as well as to understand their impact on the body with reference to haematological and biochemical parameters.

Materials & methods

Study area

Study area is semi-urban area which lies on the outskirts, towards the south-west of Dehradun. The maximum rainfall ranges between 60-80 mm during the months of July-August. The maximum temperature reaches up to 40^{o}C during the months of May and June whereas

minimum of 2^{o}C during December-January. The area of 16 sq.km around Wildlife Institute of India is also an area of high intensity of human use, having open sewage and ample garbage dumps, which is frequented by the study species as well as several others like stray cattle and dogs. However, there are distinct patches of forest cover, agricultural lands, residential area and in home range of one of the study troops, most of the communities do not have access to sustainable hygiene and sanitation facilities, which lead to human excreta ending up untreated in the environment, especially in water bodies and soil. The study area is undergoing rapid urbanization leading to increase in human density and land use, this is offering macaques high chances of interacting with humans and feral and domestic animals.

Sample collection

Collection of faecal samples: We collected Rhesus macaque faecal samples during behaviour observation. Fresh faecal samples were collected with proper-gloved hands and transferred in sterile container with 10% formalin. Collected samples were stored at 4^{o}C until lab analysis and marked with troop ID. Sample were collected during rainy season July 2021 to August 2021 (n= 52; Troop A (n= 15), Troop B (n=9), Troop C (n=18) Troop D (n=10)). Collected samples were processed same day if not then stored in 4^{o}C until lab analysis was done.

Collected faecal samples were subjected to examination, identification of parasite egg and to determine the number of eggs/gram (EPG) sample by qualitative and quantitative analysis. Qualitative analysis provides a diagnosis of both protozoal and helminthic infections and quantitative analysis helps in determination of the burden of nematode worm infections expressed in eggs per gram of faeces.

Figure 10.6 : *Collection of faecal sample from Rhesus macaque A) Fresh faecal sample B) Collected sample in 10% Formalin*

Collection of blood sample

We collected blood sample for haemato biochemical test from study troop followed by capture of Rhesus macaque. Collection of blood sample from 8 individual in yellow top vacutainer and kept for extraction of serum at room temperature as per the protocol. Serum sample were stored in -20^{o}C until lab analysis done. Serum sample were analysed in Skyla VB1 blood analyzer.

Results

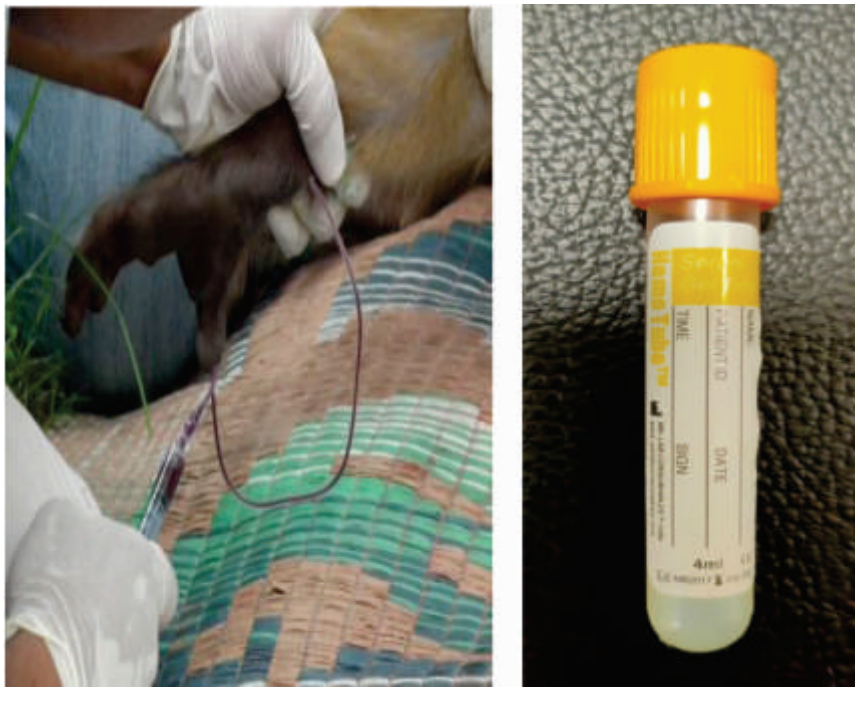
(A) Prevalence of Gastrointestinal parasites in Rhesus macaque faecal sample

We examined faecal samples processed for microscopy identification of parasite/eggs as described in methods. Nematode parasites belonging to order Strongylida, families Ancylostomatoidea, Strongyloides & Trichuridae were identified from faecal samples of Rhesus macaque. The parasite prevalence was higher in Troop-C followed by Troop-D, Troop-A & Troop-B. 4 Samples from Troop-B were found to have presence of more than one parasite species.

(i) Qualitative Analysis

A) Nematode species identified from Rhesus macaque faecal samples

Collected samples were processed as mentioned above, out of 52 samples 40 had presence of eggs/larva of nematode parasite(s). Identification of parasites was done by observing the



(A) (B)

Fig 10.7 : *Collection of blood sample from Rhesus macaque A) sample collection by veterinarian; B) Collected sample in Serum Gel tube (HemoTube™).*

morphological differences in the parasitic eggs. We were able to identify nematodes spp from *Strongylida, Strongyloides* & *Trichuridae*. Also, we were able to identify *Ancylostoma* spp from faecal samples of rhesus macaque not observed in previous study.

Ancylostoma spp.	Eggs are ovoid with thin smooth eggshell, Internally eggs are always in early mitosis, contain 2 to 16 cells (Figure 10.8)

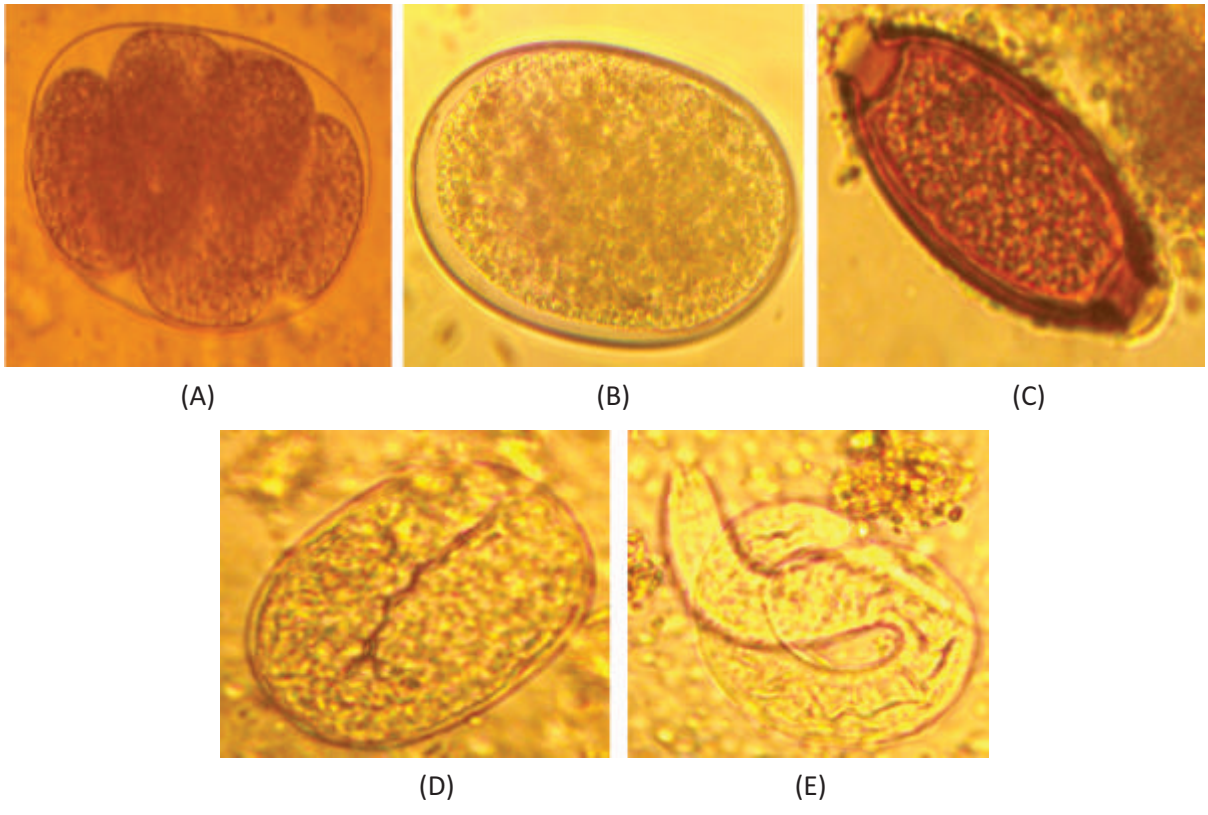


Figure 10.8 : *Identification of parasites using microscopy (A) Egg of* Ancylostoma *spp. (B) Egg of* Strongylida. *(C) Egg of* Trichuris *spp. (D & E) Egg & Larva of* Strongyloides *respectively.*

B) Prevalence of parasite species in faecal samples of Rhesus macaque in 4 study troops

Highest numbers of *Ancylostoma* spp. were recorded in Troop-B, *Strongyloides* in Troop-C, Trichuris spp. in Troop-B & *Strongylida* in Troop-C.

In microscopic observations nematode parasites belonging to *Ancylostoma* spp., *Trichuris* spp. & *Strongyloides* were found to be most prevalent in faecal samples of Rhesus macaque in all four study troops. *Strongylida* was prevalent in troop B & C. Few samples from Troop B & C were found to have multiple parasite species. Prevalence of *Ancylostoma* spp. was found only in Troop-A & Troop-C. 26.67% samples in Troop A and 16.67% samples in Troop C were found to have eggs/larva of *Ancylostoma* spp. Prevalence of *Strongylida* was found only in Troop A,B & C, with highest prevalence of 44.44% in Troop-B & 11.11% in Troop C and 6.67% in Troop A. *Strongyloides* were found in all troops with 44.44% in troop B & C, 20% in troop A and 60% in troop D. Prevalence of *Trichuris* spp. was found in two troops B &C with high prevalence of 22.22%

in Troop-C and 11.11% in Troop-B. We were not able to detect parasite egg/larvae in 12 samples.

(ii) Quantitaive Analysis

Mean of EPG & egg count in faecal samples of Rhesus macaque using Mc-master technique

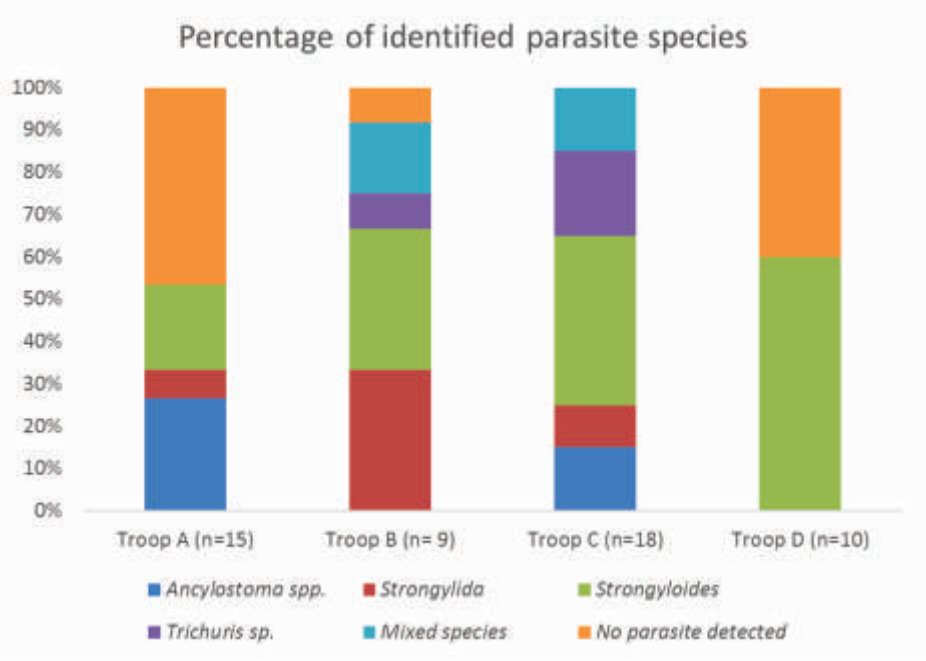


Figure 10.9 : *Parasite prevalence through direct smear technique in Rhesus macaque faecal sample from 4- study troops.*

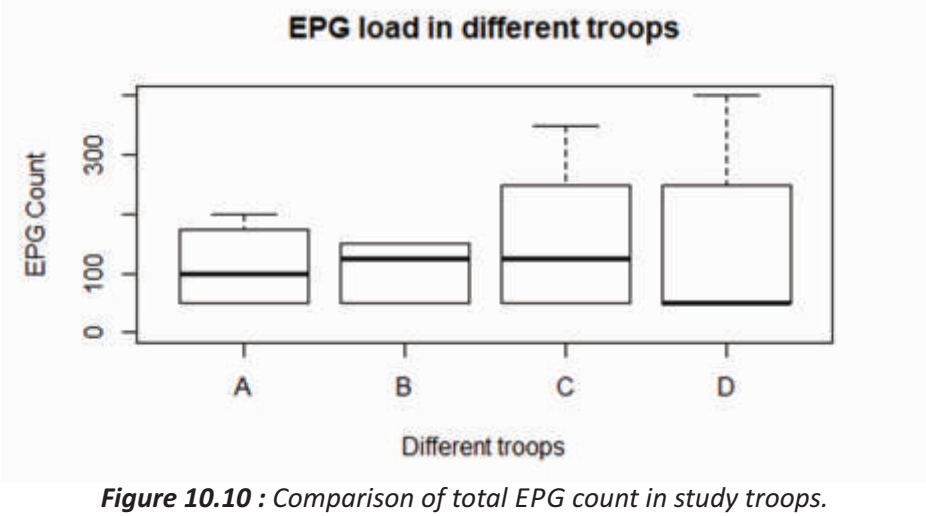


Figure 10.10 : *Comparison of total EPG count in study troops.*

Table 10.4 : Total EPG count using Mc Master from all four study troops in rhesus macaque faecal samples.

Troops	No. of eggs (Mean ± SD)	EPG load (Mean ± SD)
A	2.25 (±1.29)	112.50 (±64.95)
B	2.16 (±0.89)	108.33 (±44.87)
C	3.07 (±2.18)	153.57 (±109.32)
D	2.83 (±2.73)	141.66 (±136.67)

In the current study, Troop C was found to have high parasitic load in comparison to other troops. Animals congregating at garbage dumps are likely to acquire zoonotic diseases and spread to humans (Dobson & Foufopoulos, 2001). Home range of this falls in periphery of forest cover, garbage is disposed in open and there are no assigned garbage dumps. The garbage dumps are dynamic and other feral animals also feed on the kitchen refuse, garbage dumps have been observed to functions as additional food resources for various stray animals. Troop C inhabits forest patch as well where they interact with other animal species. Garbage dumps are the hotspots for exposure to diseases since many animals defecate there as well. Feral pigs, cattle and dogs were observed defecating at or near garbage disposal sites. These dumps may contain either infective parasite eggs or infective, free-living parasite larvae. Garbage dumps may attract many types of insects and flies, which may act as the vectors for many infectious diseases.

Least parasite prevalence was observed in troop-A macaques. Troop-A macaques have least access to garbage dumps. They mostly had seen to forage on natural plants that contain medicinal values and also have anthelmintic activity. This could be the possible reason of low parasite prevalence in Troop-A macaques.

Nematode parasites from *Strongyloides, Strongylida, Ancylostoma* spp. & *Trichuris* spp. were found in faecal samples collected from study troops. In current study, *Strongyloides* sp. were found to be most common in macaques with the prevalence rate of 52% which is higher than the findings from Devghat, Chitwan (21.4%), (Adhikari and Dhakal, 2018), India (13.0%) (Kumar et al., 2018), and lower than the findings from China (73.36%) (Zhang et al., 2019).

The high prevalence of *Strongyloides* in the study population is a source of concern, since heavy infections with this parasite have been associated with abdominal pain, bloating, heartburn, intermittent episodes of diarrhoea and constipation, a dry cough, and skin rashes. (https://www.cdc.gov/parasites/strongyloides/gen_info/faqs.html).

- **Haemato-Biochemical parameters analysed in serum samples**

Serum samples from 8 individuals were taken and analyzed in Skyla blood analyzer. Pearson product moment correlation test was performed to compare haemato-biochemical parameters with parasite prevalence. Total 14 parameters were analysed (Albumin, total protein, alkaline phosphatase, alanine aminotransferase, phosphate, amylase, blood urea nitrogen, creatinine, calcium, blood urea nitrogen/creatinine, sodium, potassium, globulin, urea, albumin/globulin & sodium/potassium) for correlation with EPG.

It was observed that ALB (Albumin) levels were lower in samples positive for parasitic presence, this decreases with the increase in parasitic prevalence is similar to observation of (Pralomkarn et al., 1997) who reported a significant fall in total serum protein and albumin in ruminants infected with nematodes. Topázio et al., 2015, also report the decrease in albumin level with the increase in parasite species of *Eimeria* spp. For a conclusive correlation more samples need to be analysed.

Table 10.5 : Haemato-biochemical correlation with parasite prevalence in free ranging Rhesus macaque

S. No.	Parameters	R Values	p Values*
1	ALB	-0.76	0.08
2	TP	-0.54	0.27
3	ALP	-0.46	0.36
4	ALT	-0.45	0.37
5	PHOS	-0.45	0.48
6	AMY	-0.54	0.44
7	BUN	0.04	0.27
8	CREA	-0.45	0.94
9	Ca	-0.37	0.36
10	B/C	-0.47	0.35
11	Na	-0.18	0.73
12	K	0.07	0.89
13	GLOB	-0.54	0.27
14	UREA	-0.46	0.36
15	A/G	-0.45	0.38
16	Na/K	0.02	0.97

10.3 Determination of Antihelmintic activity in dietary plant extract (2021-2022)

Anthelmintic plants are natural dewormers. Worms or gastrointestinal nematodes, in small ruminants and cattle can cause disease and lead to production and economic losses (e.g. poor growth rates, drop in milk yield) if not controlled.

Forage is the most important factor to correlate the prevalence of gastrointestinal parasite in Rhesus macaque. Medicinal plants have been of great interest in recent years for the management of helminthiasis due to the fact that plants often contain wide range of compounds with anthelmintic properties (Zahir et al., 2012). Most primates have a diverse plant based diet, from which they obtain the needed calories and nutrients necessary for survival and reproduction (Oats, 1987, Altmann, 1998, Lambert, 2011). The major plant dietary strategies are frugivory and folivory, supplementing this with seeds, sap, bark and flowers (Carvalho, 1996, Lambert, 2011). However, plants provide more than just nutrients, they also contain a variety of secondary metabolites that have largely been viewed as deterring animals from eating them (Glander, 1982). It has also been shown that many different primates dietary plants have both nutritional and medicinal value, suggesting that these secondary metabolites could actually be beneficial to the health of the animal (e.g. Sifaka: Carrai et al., 2003; gorilla: Cousins and Huffman, 2002; chimpanzee: Huffman, 2003, Krief et al., 2005, Krief et al., 2006; Japanese macaque: MacIntosh and Huffman, 2010). Thus, plant products containing a range of phytochemicals with anthelmintic action can often be a good choice to control helminthiasis (Mondol et al., 2015).

The objective of this study is to understand the role of dietary plants to promote self-medication and safe anthelmintic treatment including parasitic diseases in Rhesus macaque.

Materials and methods

To perform this study, we selected five different dietary plants, *Alternanthera sessilis, Ficus religiosa, Ficus palmate,* and *Shorea robusta.*

Collection, identification and processing of plant samples

Identified dietary plant samples were collected from study area following focal and behavioural observation. The plant samples were dried in hot air oven at 60^{o}C for 1-2 days and then were grinded using mechanical grinder to make a fine powder. Collected plant sample were processed to prepare methanolic and aqueous extracts.

Extraction of plant sample

The purpose of preparing plant extract is to check the difference in effects of anthelmintic properties of plants. We prepared Methanol/organic and Aqueous extract for each plant sample.

- Weigh 0.4g of powdered plant sample and soaked in 10ml of absolute methanol/ aqueous solution in ratio of 1:3 (w/v)
- Allow to stand at 27^{o}C for 48 hrs protected from light.
- Dilute the extract with PBS (phosphate buffer saline) in selected five different concentrations 0.9mg/ml, 1.9mg/ml, 3.9 mg/ml, 7.8 mg/ml, 15.6 mg/ml, to check the anthelmintic activity at different concentrations level (Cabardo Jr & Portugaliza, 2017).
- Anthelmintic activity in dietary plant species of Rhesus macaque
- For evaluation of anthelmintic efficacy in extracted plant concentration we cultured L2 stage larva from Rhesus macaque faecal sample with charcoal culture technique with following procedure.

Culture of larval stage of nematode

Procedure of culturing Nematode eggs to infectious stage larvae in laboratory aids to concentrate them more rapidly which otherwise would have taken place in soil or tissue. Culture techniques are useful for obtaining a large number of infective stage larvae.

Procedure

1. Approximately three gram of fresh faecal materials was taken and thoroughly mixed with distilled water and then mixed with equal quantity of granulated charcoal.
2. Placed the faecal-charcoal mixture at the centre of petri-dish with moistened filter paper at the bottom.
3. Petri-dish was sealed with vinyl tape and placed in a dark room (avoiding fungus growth).
4. Checked petri-dish every day and replaced evaporated water up to 7 days. On the 7th day, added water to the mixture and exposed to light for 1hour.
5. Collected Culture Water in a test tube and centrifuged for 5 minutes at 2000 rpm. Examined sediment microscopically for the presence of nematode larvae.

(A) (B) (C)

Figure 10.11 : *Charcoal culture from Rhesus macaque faecal sample (A) day1 & (B) day 10; larva microscopic of culture water, detection of L2 stage larva*

Larval motility test

Pipette 0.3ml of water from the faecal charcoal culture and examined under microscope for the presence of alive larvae.

Before introducing them in the plant extract and care should be taken that minimum around, 25-30 larvae could be present in 0.3ml of water from culture.

After checking, this quantity of water from culture added to the all-3 concentrations (0.95 mg/ml, 1.9 mg/ml, and 7.8 mg/ml) of plant extract.

For control Ivermectin and absolute methanol was taken. Left the complete setup for incubation.

Check activity of larvae after 5 hours to determine plant's anthelmintic property.

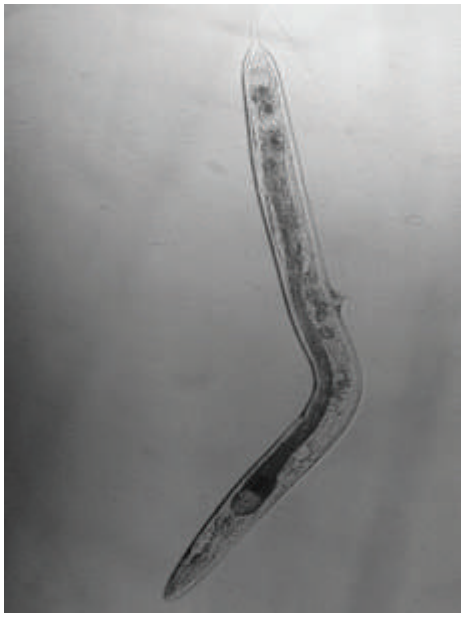


Figure 10.12 : *Dead nematode larva containing egg recovered from rhesus macaque faecal sample during larvicidal activity*

Results

Anthelmintic activity of dietary plants species of Rhesus macaque

Larvicidal activity in selected dietary plants of Rhesus macaque at different concentrations of aqueous and absolute methanol extracts.

In the larvicidal assay, the highest larvicidal activity of the absolute methanol extract was observed in plant *Ficus religiosa* at 7.8 mg/ml with 84% efficacy. Control of 0.5% ivermectin & absolute methanol was also taken, which 100% retarded the growth of larvae.

In current study, among 5 plants, *Ficus religiosa* was found to have high anthelmintic properties against nematode larvae recovered from rhesus macaque faecal samples. This observation was found to be similar with (Kaushik et al., 1981) in which he reported that *Ficus religiosa* bark methanolic extract was 100% lethal for *Haemonchus contortus* worms. It has been accepted that anthelmintic activity is due to a proteolytic fraction called ficin. It is evident from above that methanolic extracts of *F. religiosa* possibly exerted anthelmintic effect because of ficin (Hansson et al., 1986).

In current study, macaques have access to this plant, as they are largely distributed in the Chandrabani area. They are usually seen feeding on them. The leaves have been reported to have bioactive compounds (campestrol, stigmasterol, isofucosterol, tannins, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tyrosine, methionine, valine, isoleucine) which help in preventing gastric problems (Kumar et al., 2018). The methanolic, ethanolic and aqueous extracts can be made from bark, leaves and fruits (Rutuja et al., 2015). The fruits have been reported with bioactive compounds such as asparagine, tyrosine, undecane, tridecane, tetradecane, ocimene, limonene, dendrolasine, flavonoids (kaempeferol, quercetin, myricetin) and other phenolic components (Rutuja et al., 2015). All these phytochemicals have been reported to exert medicinal properties such as anti-bacterial, anti-diabetic, anti-convulsant, anti-amnesic etc (Kumar et al., 2018).

Among 3 concentrations (0.95 mg/ml, 1.9 mg/ml, and 7.8 mg/ml) in methanolic extract of *Ficus religiosa* plant, highest larvicidal activity was recorded in 7.8 mg/ml concentration. The solvent used for plant extraction can also greatly influence the chemical composition of the extract (Kalt et al., 2001). The highest extraction yields were recorded by methanol for decoction and acetone for maceration. The total polyphenol content (TPC) obtained by decoction had the highest TPC contents, and MeOH containing NaF was the best solvent for the extraction of TPC (Lezoul et al., 2020).

Due to the high concentration of organic compounds in plant, extraction using methanol was efficient. It was observed that in extract concentration 7.8mg/ml high larvicidal activity was recorded, all the bioactive compounds might have been extracted very well at this concentration.

Asian Elephant

10.4 Disease Ecology

Although humans have always shared habitats with wild animals, the dynamics of this interactions have radically changed. Over the last few decades, the world has witnessed radical changes in climate, landscape, and ecosystems. These events, together with other factors such as changing human behaviour towards wildlife, are resulting into thinning boundaries between wild animals, their domestic counterparts and humans. Wild animals worldwide are now being increasingly found in proximity to humans and their domestic animals, creating a potential environment for disease spillover. Thus, an underemphasized yet important subset of human wildlife conflict is zoonoses and its implications on public health. For example, Among the spectrum of pathogen harbored by macaques, enterobacterial pathogens including *Salmonella spp.,* and *Escherichia coli* are the most distributed pathogens in non-human primates and are often transmitted to human and domestic animals by water sources and environment contaminated with faeces (McLennan et al., 2018). Several studies have also documented tuberculosis in Rhesus macaques, both in captivity and free ranging populations alike (Payne et al., 2011; Rothschild, 2015; Shrestha et al., 2017). Infectious diseases may also be particularly of relevance to free-ranging wildlife health. Because of radical changes in ecosystem, the epidemiology of diseases caused by a number of infectious agents is undergoing profound readjustments, as pathogens adapt to new hosts and environments. Since domestic animals are maintained at high population densities and have a near-global distribution, they are known to serve as reservoirs of infectious disease for wild mammals (Lafferty & Gerber 2002; Fiorello et al., 2004). For example, a *Mycobacterium tuberculosis* surveillance program by Zachariah and co-workers (2017) in free ranging elephants of Kerala indicated possible spillover of the agent from humans/domestic animals in the study area to wild elephants. A similar study by Jethva and Jhala (2004) on wild canids of Gujarat found widespread prevalence of rabies and canine distemper in study species, with rabies being a major source of mortality amongst adult Indian wolves, golden jackals and domestic dogs in their study area. Hence, the identification of the hazards arising from the co-habitation is critical in order to plan and develop adequate control strategies against these pathogens. Since current project involves extensive biological sampling from study species across various interaction levels (captivity and free ranging) for hormone and genetic studies, it also provided a unique opportunity to study certain diseases, helping in better understanding of both the drivers of emergence at the interface, as well as effect of such diseases on study species.

Anti-Microbial Resistance Studies

Isolation and characterisation of antimicrobial resistant Enterobacteriaceae

The neglectful usage of antibiotics in agricultural, animal husbandry and medical sector has led to the development of antibiotic resistance which has become a global problem. Massive evidence attests the occurrence of anti-microbial resistance (AMR) bacteria exchanges

between human, wildlife and domestic animals (Vittecoq et al. 2016). Evidences suggests water as main transmission media (Hame- lin et al. 2006; Galvin et al. 2010; Dhanji et al. 2011; Taylor, Verner-Jeffreys & Baker-Austin 2011; Zhao & Dang 2012). The intimate relationship between camp elephants and their mahouts and the subsequent interaction of camp elephants with wild elephants establishes opportunities for cyclical transfer of AMR in between these three entities. Thus, to understand the interaction and development of AMR at the interface, camp elephants from Karnataka Forest Department were studied as described below.

Methodology

Sample Collection: Rectal swabs were collected in BHI Broth and stored at 4^{o}C before processing.

Media used for isolation of pure cultures: Brain Heart Infusion Broth (BHI), Rappaport Vassiliadis Broth, Luria Bertani Broth (LBB), MacConkey Agar (MLA), Eosin Methylene Blue Agar (EMB), Hektoen Enteric Agar (HEA), Salmonella Shigella Agar (SSA), Brilliant Green Agar (BGA)

Methodology for culturing: A sterile inoculation loop was inserted into the feacal sample and enriched in BHI broth and Rappaport Vassiliadis Broth separately followed by 18-24 hours of incubation at 37^{o}C. Subsequently BHI enriched cultures were streaked on MLA and Rappaport enriched cultures on SSA and incubated for 18-24 hours at 37^{o}C. Further separate colonies were isolated from the mixed culture on MLA and grown on EMB, HEA, BGA and incubated for 18-24 hours at 37^{o}C. Single colonies from pure cultures hence isolated were stored in glycerol (50%) for further molecular studies.

Antibiotic Sensitivity Test: The antibiotic susceptibility profile of the Gram-negative isolates was determined using the standard Kirby-Bauer disk diffusion method. These antibiotics with their respective disk concentrations are as follows:

Penicillins	Ampicillin (10mcg), Penicillin(10mcg), Amoxycillin (30mcg)
Cephalosporins	Ceftazidime (30mcg), Cefotaxime (30mcg30mcg), Ceftriaxone (30mcg)
Carbapenems	Doripenem (10mcg), Meropenem (10mcg), Ertapenem (10mcg),
Aminoglycosides	Amikacin(30mcg), Gentamicin (10mcg)
Quinolone	Ciprofloxacin (5mcg)
Polymyxin	Colistin (10mcg)

Antibiotic Sensitivity Test: Bacterial culture suspension was spread on Muller-Hinton agar plates using L spreader and a maximum of 6 antibiotic disks were used on a single petri plate and incubated at 37^{o}C for 18-24 hours; then, the inhibition zone diameters around the antibiotic disks were measured. The results were expressed as susceptible or resistant according to the criteria recommended by the CLSI. As per plan, the phenotypic study will be followed by molecular confirmation of resistant genes present in these isolates based on the phenotypic study results.

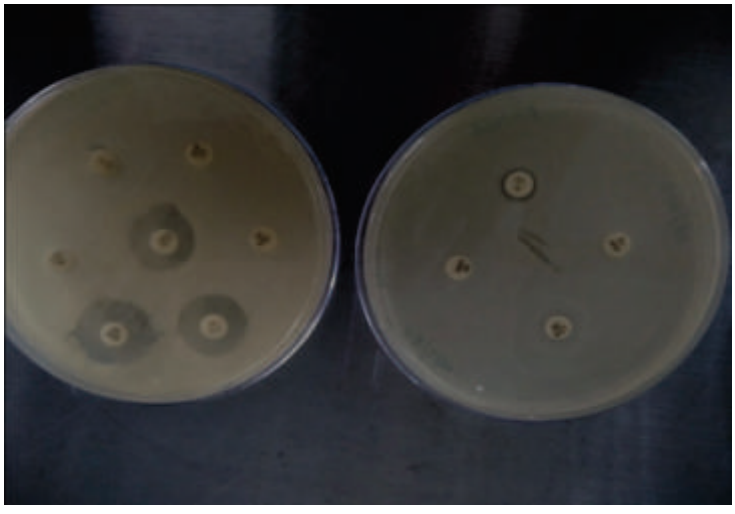


Figure 10.13 : *Elephant sample showing resistance towards antibiotic.*

Double Disk Synergy Test: Isolates which were resistant to more than three classes of antibiotics were tested for the confirmation of ESBL. Test isolates were spread on Mueller Hinton Agar plates following the same method used for AST. Amoxicillin, ceftazidime and cefotaxime were chosen for DDST with their respective combination drugs amoxicillin/clavulanic acid (20/10mcg), ceftazidime/clavulanic acid (30/10mcg), cefotaxime/clavulanic acid (30/10mcg). Each antibiotic was placed with its combination drug in each plate incubated at 37^{o}C for 18-24hours as per the CLSI guidelines.

Results : A total of 27 representative elephant rectal swabs from camp elephants have been processed so far. While all the 27 samples are suspected to be *E.coli* positive based on culture results, 14 samples are suspected to be *Proteus sp.* positive. For Results of AST, 18 samples were intermediate to resistant for ceftriaxone(30mcg), 10 samples were intermediate to resistant for Ciprofloxacin(5mcg), 5 samples were intermediate to resistant for gentamicin(10mcg), while 3 samples were completely resistant to Ampicillin(10mcg) and Amoxycillin (30mcg). After confirmation of ESBLs producing isolates were subjected to PCR.

Detection of resistant genes: For Genotypic characterization of AMR, DNA was extracted from 21 phenotypically resistant isolates and subjected to multiplex PCR with primers that correspond to conserved regions of beta lactam encoding genes- blaTEM, blaSHV, blaCTX-M and blaIMP. Out of 21 samples blaSHV was detected in 7 isolates, blaIMP in 4, blaCTX-M and blaTEM in 1 isolate.

Screening of Camp Elephants for Endotheliotropic Herpes Virus (EEHV)

EEHV is an acute highly fatal haemorrhagic disease in both wild and captive young elephants, with EEHV1a and EEHV1b being the predominant strains in Asian elephants. The disease is of grave concern worldwide as it is regarded to significantly impact elephant population sustainability (Angkawanish et al. 2019) as they are causing mortality not only in captivity but also has been detected in wild (Zachariah et al. 2013). Nonetheless, there is an acute paucity of information on the prevalence of the disease even from range country such as India. Through

this project, blood collection opportunities in captive elephants, coupled with trunk wash collection were utilised to collect samples to screen for EEHV.

Methodology

Sample Collection and Transport: Blood samples and trunk washing samples were collected from the different camp elephants of Karnataka. The camps sampled were located at Mathigodu, Rampura, Dubare, Anekadu, Doddaharve, Sakrebyle, Hebbale, B Colony, Balle and Mysore palace. Two dead wild elephants were also samples there. The time of sampling ranged from December 2019 to November 2020. Over this period a total number of 99 blood samples have been collected in EDTA vacutainers and some with 0.5ml DNA/RNA shield. All samples were aliquoted from the main collection vial and stored at -20^{o}C prior to processing.

DNA extraction: DNA extraction from blood was carried out using the DNeasy Blood and Tissue lit (Qiagen) with the kit protocol for blood. For trunk washing samples DNA was extracted using the DNeasy Blood and Tissue lit (Qiagen) with slight modifications devised by the Baylor College of Medicine based on the DNeasy Blood and Tissue spin protocol for tissues.

PCR: Screening of EEHV in the blood and trunk washing samples were done using nested PCR with the set of two-round PAN-EEHV Pol locus primers 6710/6711/6712 (Latimar,2011). After the primers were initially standardized by running gradient PCR.

Gel Electrophoresis: The PCR products are subjected to gel electrophoresis using 2% agarose gel stained with Sybrsafe (Invitrogen). The amplicon sizes were compared using 100bp ladder. The expected amplicon size after the first (6710/6711) and second rounds (6710/6712) of amplification were 500bp and 250bp respectively.

Sequencing: Sanger sequencing have been carried out on positive samples.

Results: Out of the total 99 samples tested for PAN-EEHV POL locus primers, 24 tested positive and have been subjects to DNA sequencing. Interestingly, two wild elephant samples have also been tested positive confirming that EEHV is not only in captivity but also prevalent in wild.

10.5 Gastrointestinal Parasite Studies in Asian Elephant

Gastrointestinal helminths are common in all animals (e.g. Fagiolini et al. 2010). Nematode worms and particularly strongyles are prevalent among mammals and their control is an important aspect of animal husbandry. While internal parasites of domesticated species are well documented, studies on gastrointestinal helminths in species such as elephants, both in captivity and in the wild, are limited (Woodroffe 1999). The Asian elephant (*Elephas maximus*) is classified 'endangered' (IUCN 2017). Asian elephants are unique among endangered megafauna in that a significant number are in captivity and managed by a variety of institutions and individuals.

Parasitism affects both evolution and ecology of host species through sexual selection

(Hamilton and Zuk 1982) and parasite mediated competition results in reduced population size or extinction (Price et al. 1986). Generally, endoparasites are not life threatening in healthy adult animals (Hing, 2012). However, changes in disease dynamics due to altered host, environment or pathogen factors can result in clinically significant endoparasite infection (Hing, 2012). Even normally innocuous parasites may diminish individual and population health, reproductive success and fitness when combined with other threats including concurrent disease, malnutrition, significant stressors, lowered immuno competence and decreased genetic variability (Lloyd, 1995). Decreased genetic variability reduces a host population's ability to cope with infectious disease and parasitism by decreasing average resistance and/or reducing variations in adaptive traits (Scott, 1988; Luquet et al, 2011).

Nutritional and physiological status, stress and captive conditions of animals can influence their resistance to parasites (Geraghty et al. 1982). It has been found that the severity of parasite infections is higher in weaker animals as compared to healthy animals (Lively & Dybdahl 2000; Smith et al. 2009).

The elephant is unique regarding most of its internal (and a few external) parasites not being shared with other livestock hosts. According to published literature, the Indian elephant is parasitized by 39 helminths, consisting of 8 trematodes including the most pathogenic *Fasciola jacksoni*, 2 cestodes and 29 nematodes including 3 filariid worms; among protozoa, only *Trypanosoma, Babesia* and ciliates are known; arthropods include the stomach bot *Cobboldia,* the louse *Haematomyzus* and at least 4 genera of ticks (Pathak and Chhabra, 2012).

Occurrence of parasites in captive elephants is reported to vary according to husbandry practices, disease prophylaxis and treatment (Fowler 2006; Vanitha et al. 2011).

Parasitic infections can cause diseases and death in wild animals and can become a source of infection for domestic animals and vice-versa. Epidemiological studies are essential to know the status and transmission of such diseases. Parasitic diseases in domestic animals living in vicinity of wildlife are best controlled by preventing contact between wild and domestic animals and by manipulating the factors involved in disease transmission. Further, anthropogenic influence on habitat results in increased parasite prevalence in Bornean elephants (Hing et al. 2013). Estimation of that at least half of all known species are parasitic, so understanding the life cycle and interaction of these organisms with their hosts is often key to understanding the dynamics of ecosystem.

Present study was initiated in Karnataka to assess gastrointestinal parasites in wild elephant and semi captive elephants with objectives-

(1) To identify different endoparasites, estimation of parasitic load and to evaluate feasibility of using parasite egg morphology to identify parasite species.

(2) Correlation between haemato-biochemical and parasite prevalence on the health of semi-captive Asian elephants.

10.5.1 Prevalence of Gastrointestinal parasites in Asian elephants (semi captive and wild elephants)

Over a period of 30 years, Chandrasekharan and his colleagues at the Kerala Agricultural University Elephant Research Centre surveyed captive and wild elephants. Using unspecified parasitological methods, they found 21 different species of helminths. Prevalence of helminths was reported to be high; all captive elephants and 38% wild elephants harboured strongyles (Chandrasekharan et al., 2009). Vidya and Sukumar (2002) reported using sedimentation-flotation method, a similar inventory of endoparasites in their study on free ranging wild elephants in southern India.

Chandrasekharan et al. (2009) and Saseendran et al. (2004) reported that gastrointestinal nematode infection has been associated with frequent clinical illness including colic, diarrhoea and dependent oedema in elephants managed in captivity in Kerala, India. In the extreme, case reports exist of elephant fatalities associated with endoparasites.

Parasite prevalence is reported to be high in wild elephants, some camps are maintaining elephants in a semi-captive state i.e. they are allowed to roam around the adjacent forest areas and return to camp in the evening. Since semi-captive elephants visit forest areas, they have high chances of interacting with wild elephants for mating, socializing and sharing natural resources. Semi-captive elephants receive deworming treatments regularly; we are investigating the prevalence of gastrointestinal parasites in the wild elephants and the semi-captive elephants interacting with them. Whether some particular endoparasites (gastrointestinal parasites) are specific to the two elephant groups. Further how deworming treatments influence the parasite load and if infested animals display some clinical signs of disease (haemato-biochemical assessment).

10.5.2 Haemato-Biochemical studies and Prevalence of parasite in semi captive elephants

The objective of this study is to know the prevalence of parasitic species of the Asian elephants as well as to understand their impact on the body with reference to haematological and biochemical parameters. Haematological findings of affected elephants with non-significant low haemoglobin, packed cell volume and total erythrocyte count suggested anaemic condition on comparison with healthy elephants, which substantiate the findings of anaemia by Sarode et al. (1991). Study was carried out in semi-captive elephants only to correlate haemato-biochemical value with parasite prevalence.

Materials and methods

Study area

The study included both wild and semi-captive elephants from Kodagu district, Karnataka, Situated on the top, the eastern and western slopes of the Western Ghats, Kodagu district occupies about 4,102 sq. km (1,580 miles) area of the Ghats on the South Western border of Karnataka, i.e., between 11^{o}56' and 12^{o} 52'N latitudes and 75^{o}22' and 76^{o}11'E longitudes.

The study on semi captive elephant was carried out in Rampura and Mathigodu camp, located in Aanechowkur range of Nagarahole Tiger Reserve. These camps are mostly in idyllic extensive conditions of free ranging, have scope for interactions with other elephants, bathe in rivers and lakes and breed with both camp and wild elephants. Numbers of elephants, maintained in a semi-natural condition, with plenty of access to forest and rivers/streams and opportunity to interact with wild elephants (Verma et al., 2008). The diet in the camp includes, cooked finger millet and rice balls, paddy tied together in paddy straw (Kusure in local language), salt, coconut and jaggery; apart from the natural foliage/feed which elephants consume during their foraging period in Forest (Verma et al., 2009).

The study on wild elephants was carried out in Virajpet forest division, which occupies the southern part of Kodagu Forest circle.

Collection of dung sample

For semi-captive elephants, fresh dung sample was directly taken from the elephant with proper-gloved hand by veterinarian. Collected sample was transferred to sterile container with 10% formalin.

We collected wild elephant's dung, during transect walks and samples were collected with proper gloved hands and transferred in sterile container with 10% formalin. Collected samples were stored at 4^{o}C until lab analysis. Samples from semi captive elephants were marked with the approximate age and sex of the animal. For laboratory analysis, samples were transported to Wildlife Institute of India, Dehradun as per the protocol.

(A) (B)

Figure 10.14 : *Collection of Dung sample from semi captive Elephant A) sample collection by veterinarian; B) Collected sample in 10% Formalin.*

Collected dung samples were subjected to microscopic examination for identification of parasite eggs and to determine number of eggs/gram sample (EPG) by qualitative and quantitative analysis respectively. Qualitative analysis provides a diagnostic method for the diagnosis of both protozoal and helminthic infections and quantitative analysis provides a quantitative determination of the burden of nematode worm infections expressed in eggs per gram of faeces/dung.

For semi captive elephant we collected fresh Dung sample containing formalin in winter season in December 2019 (n=25). For wild elephant we collected fresh Dung sample containing formalin in winter season on January 2021 (n=31).

Microscopy was performed using Magnus MLX Plus microscope with Magnus Camera and optika software.

Collection of blood sample

25 blood samples were collected in yellow top vacutainer from the cephalic vein of each semi-captive/ camp elephant from Mathigodu (n=16) and Rampura (n=9) camp. Blood samples were stored at room temperature for extraction of serum as per the protocol for haemato-biochemical analysis. Serum samples were stored in -20^{o}C until laboratory analysis. Serum samples were analysed in skyla VB1 blood analyzer.

(A) *(B)*

Figure 10.15 : *Collection of Blood sample from semi captive Elephant*
A) sample collection by veterinarian; B) Collected sample in Serum Gel tube (HemoTube™)

Laboratory analysis

Parasite prevalence in Asian Elephant (Semi captive and wild elephants)

Qualitative analysis

For the observation of parasite eggs in the dung sample, floatation and direct fecal smear methods were performed.

Direct smear Technique

This technique provides a quick & simple but relatively intensive method for demonstrating helminth infection and identifying the eggs and larvae present.

Procedure

- Small quantity of sample was mixed with few drops of water, then smeared on the clean microscope glass-slide.
- Placed the coverslip over smear and then observed under microscope at 10x magnification

Floatation technique

This technique is sensitive for the detection of helminths and protozoans based on using an emulsifying liquid of greater specific gravity than that of parasite eggs, which results in the flotation of eggs in the solution.

Procedure

- For floatation technique, used saturated solution of NaCl as an emulsifying fluid. To Prepared solution, we took 400g of NaCl was dissolved in 1000ml of distilled water and stirred until the clear solution was obtained.
- Then with gloved hands, weighed 2g of fecal sample and added 30ml of saturated NaCl solution in a container and mixed well.
- The well-mixed slurry sieved through 1mm strainer and fibrous contents of fecal samples were discarded. The filtrate was transferred to a test-tube and filled to the brim to create a positive meniscus.
- A coverslip was then gently placed on the test-tube and the set-up was left to stand for 15-20 minutes for allowing the eggs to float on the surface.
- After this, the coverslip was gently removed from the top of the test-tube in such a way that drop of the fecal solution was hanging on the coverslip.
- This was then placed on the clear glass-slide and observed under the compound microscope at 10x magnification.
- Scanned every area of slide until all the fields of the coverslip were covered which took upto 20-30 minutes per sample.

- All Parasites eggs were photographed and measurements were taken using microscope camera software for identification.

Quantitative Analysis

The Mc Master technique is used for counting helminth eggs in fecal samples. This technique uses a counting chamber, which enables a known value of fecal suspension (2× 0.15ml) to be examined microscopically.

Procedure

- Approximately 2g of faecal sample was taken and dissolved with 14 ml floatation solution in a container thoroughly mixed and strained.
- Filter the suspension through strainer in another container and sub sample is withdrawn using Pasteur pipette for filling both of chambers of a McMaster slide.
- Allowed the slide to rest for 5 minutes until the eggs floated near the surface of the slide for easier detection, and was then observed under 10x magnification lens.
- The eggs within the counting chamber were counted and the total of both the chambers was added. This sum of the eggs of the 2 chambers was then multiplied by 50 to obtain the egg per gram count of the faecal sample.
- EPG= N * 50 (EPG- eggs/gram fecal/dung sample; N- no. of eggs counted in 2 chambers of Mc masters slide)

Haematobiochemical Examination

For haematological examination, the biochemical analysis including serum creatinine, blood urea nitrogen (BUN), alanine transaminase (ALT) and alkaline phosphatase (ALKP) which was carried out with ready to use kits using automatic analyser (SKYLA VB1 blood analyzer).

The Skyla Basic Biochemistry Panel used with Skyla Clinical Chemistry Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Alanine Aminotransferase (ALT/GPT), Blood Urea Nitrogen (BUN), Creatinine (CREA), Blood Glucose (GLU), Total Protein (TP), Uric Acid (UA) in human whole blood, plasma, or serum.

Clinical significance of Haemato-Biochemical parameters

Albumin (ALB) ALB is the major protein component of normal serum, accounting for more than 50% of the total protein. It plays an important role in the regulation of the osmotic blood pressure. Abnormal ALB values may be caused by dehydration, malnutrition, nephrotic syndrome or liver dysfunction.

Alanine Aminotransferase (ALT/GPT)

ALT is one of the indicators of liver function. Acute and chronic hepatitis, drug induced liver injury, fatty liver, cirrhosis, myocardial in fraction, myocarditis and biliary diseases can lead to elevated ALT activity.

Blood Urea Nitrogen (BUN)

BUN is one of the important markers for diagnosis and prognosis tracking of kidney diseases and metabolic disorders. Other common possible causes of elevated BUN include dehydration and heart failure.

Creatinine (CREA)

CREA is the degradation product of creatinine in human muscles. It is a commonly used marker to examine renal functions. Elevated CREA in the blood may be caused by severe muscle disease, nephritis, hyperthyroidism and malnutrition.

Glucose (GLU)

GLU can be used for the diagnosis of diabetes and diseases related to the carbohydrate metabolism. Diabetes, chronic pancreatitis and certain endocrine diseases may lead to hyperglycemia. Abnormal glucose metabolism, islet cell tumors, pancreatic tumors and severe liver diseases may lead to hypoglycemia.

Total Protein (TP)

TP is an indicator for the liver function and kidney diseases. Elevated TP could be caused by dehydration or increased immunoglobulin levels. And TP reduction may occur in the disorders include malnutrition, nephrotic syndrome, various liver diseases and malignant tumors.

Uric Acid (UA)

UA can be used for diagnosis and prognosis trackingof kidney related diseases and diseases caused by metabolic disorders. Kidney diseases, lactic acidosis, dehydration, preeclampsia, or diabetic ketoacidosis can lead to increased UA concentration.

Albumin/Globulin Ratio (A/G Ratio)

The A/G Ratio is the ALB and GLOB ratio. It is used to assess liverfunction and is an important indicator for the diagnosis of viral hepatitis and cirrhosis.

Estimated Glomerular Filtration Rate (eGFR)

eGFR is the kidney filtrate per minute, which is calculated from CREA. It is used to assess renal function.

Globulin (GLOB)

It is calculated from TP and ALB. It is used to assess liver function.

Test Steps

1. Open the aluminium pouch and remove the reagent disc.
2. Remove the diluent container sealing.

3. Using a micropipette to inject 200µL of the sample into the reagent disc through the sample port.
4. Place the reagent disc to the analyzer drawer.
5. Press the "start" button on the screen to initiate testing.

Note:

1. To operate the reagent disc or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.
2. The used reagent disc, tips should be discarded as biomedical waste.
3. Testing should be performed within 20 minutes after the pouch is opened.
4. Do not place the reagent disc at the environment more than 25^{o}C and longer than 48 hours prior to use.
5. If the reagent disc or its package is damage or is over the expiry date, do not use it.

Results & Discussion

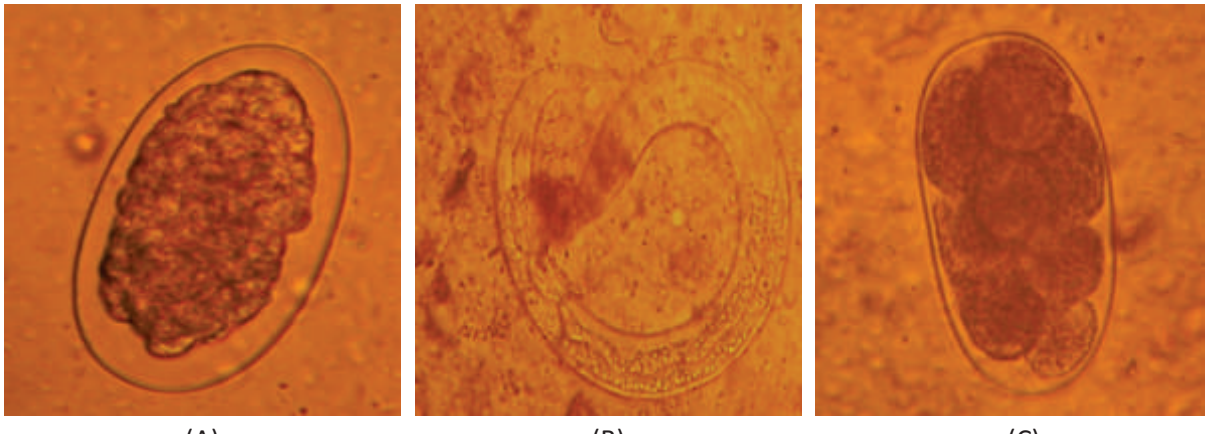
Prevalence of Gastrointestinal parasites in Asian elephants (semi captive and wild elephants)

We examined dung samples processed for microscopy for identification of parasite/eggs as described in methods. Nematode parasites belonging to *Ancylostoma* spp. & *Strongyloides* were identified from dung samples of Asian elephants. The parasite prevalence was significantly higher in wild Asian elephants.

(i) Qualitative Analysis

A) Parasites species identified from Asian elephant (wild and semi-captive) dung samples

The identification of parasites was done by observing the morphological differences in the parasitic eggs as tabulated below. Only two parasite species (*Ancylostoma* spp. & *Strongyloides*) were identified from dung samples of Asian elephants.



(A) (B) (C)

Figure 10.16 : *Identification of parasites using microscopy A) Egg & B) Larva of* Strongyloides*; C) Egg of* Ancylostoma *spp.*

Table 10.6 : Characteristic feature of Parasites found in Asian elephant dung sample.

Strongyloides	The identification pointer to this family is the presence of a L2 stage of larvae within the egg (Figure 10.16)
Ancylostoma spp.	Eggs are ovoid with thin smooth eggshell, internally eggs are always in early mitosis, contain 2 to 16 cells (Figure 10.16)

B) Prevalence of parasite species in dung samples of semi captive and Wild Elephants

In microscopic observations two parasite species *Ancylostoma* spp. & *Strongyloides* were found to be most prevalent in dung samples of Asian elephant (wild and semi-captive). For dung samples of semi-captive elephants (n=25), *Ancylostoma* spp. were found in 8%, *Strongyloides* in 40% and in 52% of the dung samples no parasite eggs/larva were detected. In wild elephants (n=31) all samples were positive with parasite egg and only one type of parasite species *Ancylostoma* spp was found in all samples.

(ii) Quantitaive Analysis

Mean of EPG & egg count in dung samples of Semi-captive and wild elephant using Mc-master technique

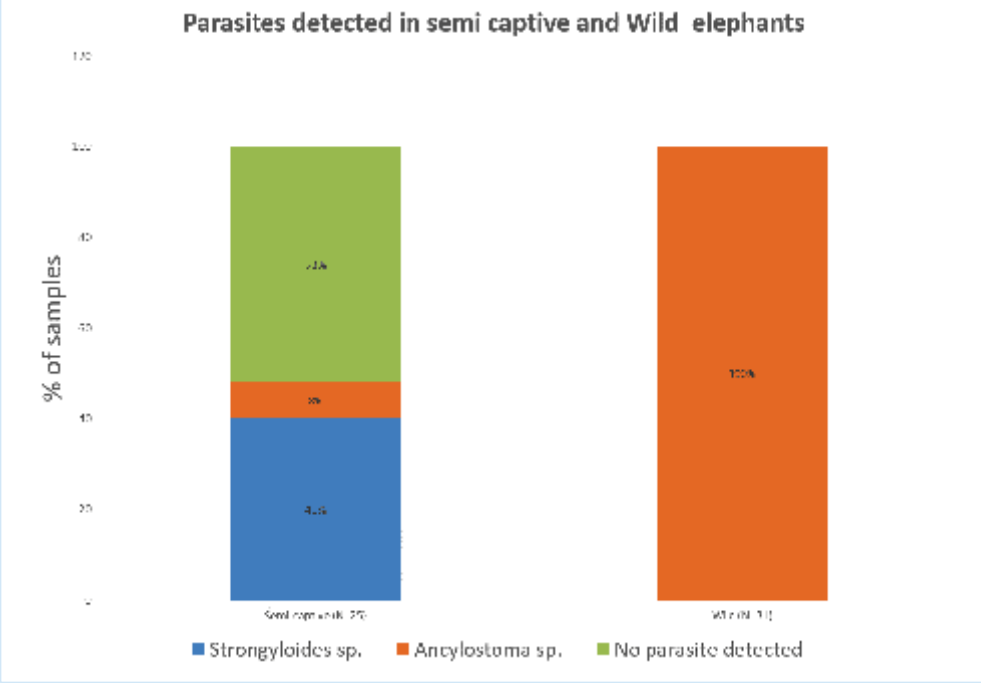


Figure 10.17 : *Parasite species percentage found in semi captive and wild elephants*

Table 10.7 : Total EPG count using Mc Master between semi-captive and wild elephants dung samples.

Samples	EPG load mean (±SD)	No. of eggs mean (±SD)
Semi-captive elephants	75 (±53.45)	2.35(±2.97)
Wild elephants	194.23 (±117.93)	2.96 (±2.41)

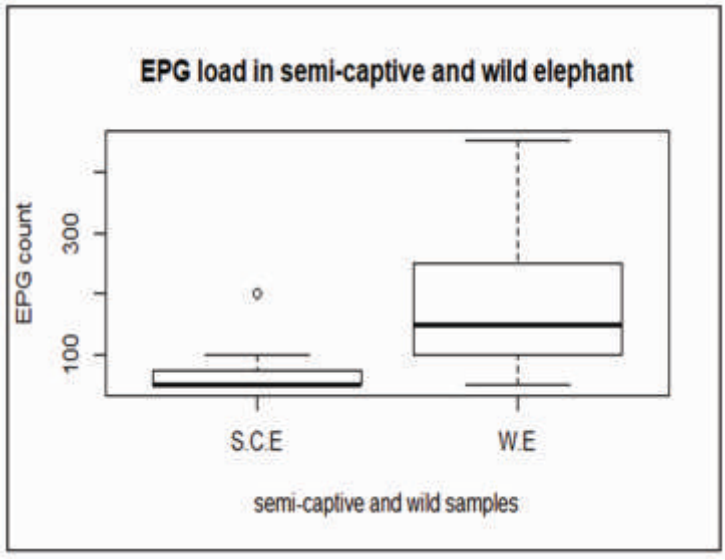


Figure 10.18 : *Box-plot for comparison of total EPG count between semi-captive and wild elephants.*

Total EPG load estimated by Mc-master technique was significantly higher in dung samples of wild elephants as compared to the total EPG load in dung samples of semi-captive elephants (Mann-whitney U-test was performed where, W = 34.5, P-value = 0.00412).

In the current study, parasite prevalence was determined between semi-captive and wild elephants. Wild elephants found to have high parasitic load in comparison to semi-captive elephants. This result was similar to the findings of (Abeysekara et al., 2018) in which authors report the infection in wild elephants (93.3%) was significantly higher than that of the captive (55.0%) and semi-captive elephants (25.0%). Unlike the wild elephants, the captive and semi-captive elephants receive regular deworming which is the main reason for having a low prevalence of GI parasites in these two groups. In general, oral deworming treatments are given with special treatments if elephants show clinical symptoms. The prevalence of helminth infections was higher in wild elephants than the other two groups. This could be because the anthelminthic drugs mainly target the helminthes (Abeysekara et al., 2018)

All the wild elephants sampled from Virajpet forest division were infected. Due to water scarcity in these areas, many elephants congregate and depend on a single water hole. This tends to increase the contamination rate as they defecate on the ground and there is a higher possibility of infection of the whole herd when one individual is infected. Potential factors determining the transmission of GI parasites in the wild include environmental conditions that affect the viability and behaviour of parasite propagules, as well as feeding, movement, and defecation patterns of the host, which determine the parasites encountered (Watve 1995; Vidya & Sukumar 2002).

The high prevalence of *Ancylostoma* spp. in the study population is a source of concern, since heavy infections with this parasite have been associated with abdominal pain or other gastrointestinal symptoms during early infection. Later iron deficiency may develop because of chronic blood loss. Hookworms are major cause of iron deficiency anaemia in endemic regions

(https://www.msdmanuals.com/en-in/professional/infectious-diseases/nematodes-roundworms/hookworm-infection) *Ancylostoma caninum* can establish pathogenic human infections dependent on the migration of L3 to the ectopic site in the human host (Mahdy et al., 2012). Hookworm is generally transmitted actively by its infective larvae which penetrate through the skin; however, passive transmission can occur through contaminated food or soil that is licked or swallowed by the host. Hookworm theoretically has a two-way transmission of the infective stage, which could account for the high prevalence recorded in this study once the infective stages are dispersed in soil. The dispersal is facilitated due to defecation in open areas, the parasite stages in the faeces are dispersed in the environment leading to the easy transmission (Larbi et al., 2020). In semi-captive elephants, high prevalence of *Ancylostoma* spp. was recorded. This observation was similar in the findings of (Abhijith et al., 2018).

Haemato-Biochemical parameters analysed in serum samples of semi- captive elephants

Total 14 parameters were tested (Albumin, total protein, alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, creatine phosphokinase, blood urea nitrogen, calcium, phosphorus, sodium, potassium, chloride & magnesium) for correlation with parasite infestation. Serum samples from 15 semi-captive elephants were analysed for the above-mentioned parameters. Serum albumin showed a significant decline (R=0.64, P= 0.01, Pearson product moment correlation test).

This observation is in line with Ali et al., 2013 in which he reported *Eimeria necatrix* infection in chickens caused a reduction in the levels of total protein, albumin and globulin, as well as an elevation in cholesterol concentration in serum.

Table 10.8 : Haemato-biochemical correlation with parasite prevalence in semi-captive elephants.

S.no	Parameters	R Value	p value
1	ALB	0.64	0.01
2	TP	-0.08	0.76
3	ALP	0.21	0.00
4	AST	0.83	NA
5	GGT	NA	0.24
6	CPK	-0.31	0.96
7	BUN	0.01	0.52
8	Ca	0.17	0.72
9	PHOS	-0.10	0.74
10	Na	0.09	0.26
11	K	-0.30	0.60
12	Cl	0.14	0.44
13	Mg	0.21	0.15
14	GLOB	-0.37	0.96
15	UREA	0.01	0.02
16	A/G	0.58	0.05
17	Na/K	0.49	NA

SECTION V

Reproductive Biology of Conflict Species

Chapter 11

REPRODUCTIVE BIOLOGY OF STUDY SPECIES

Priya Gusain, Bhavana Sahu, Thammaiah Chekkera Kuttappa, Lallianpuii Kawlni, Sanath Krishna Muliya Kafil Hussain, Vishnupriya Kolipakam, Qamar Qureshi

11.1 Reproductive Endocrinology

Analysing steroid metabolites in faecal samples represent a powerful and non-invasive tool for a broad range of research fields such as wildlife endocrinology, animal welfare, ecology and reproduction (Sheriff et al., 2011; Palme, 2012; Dantzer et al., 2013; Schwarzenberger & Brown, 2013). Metabolism and excretion of steroids differ widely between species and even between sexes (Touma et al. 2003; Palme et al., 2005) and faecal steroid metabolites are a mixture of several metabolites with different structures and polarities. Steroids are heavily metabolized by the liver and in the gut (Möstl and Palme, 2002) and although they are present in the blood, native, unmetabolized glucocorticoids such as cortisol or corticosterone are virtually absent in the faeces. The same is true for other steroids such as progesterone and testosterone (Palme et al., 1997; Dantzer et al., 2011) as verified by almost all radiometabolism studies conducted so far (for a review see Palme et al., 2005), which report only very small amounts, if any, of radioactive substances with chromatographic properties similar to the parent steroids in the blood. In most cases radiolabelled steroid hormones (e.g., 3H/14C-cortisol/-corticosterone or -progesterone) have been added to faecal samples to estimate the efficiency of extraction procedures (Young et al., 2004; Ziegler and Wittwer, 2005). The results do not reflect the actual recoveries (Möstl et al., 2005; Palme, 2005).

Although measurement may appear straightforward and easy but there is a strong need to validate these non-invasive methods extensively for each species (Touma and Palme, 2005) and for each steroid of interest.

Sex ratio are among the most basic demographic parameters which provide an indication of both the relative survival of females and males and future breeding potential of a population. One of our objectives is to study reproductive cyclicity in females via estimation of reproductive hormones in collected faecal and blood (serum) samples by using commercially available enzyme immunoassay kits, collection of samples performed frequently at fixed intervals. To understand the reproductive physiology of wild Rhesus Macaque population studies on estrous cycle of female animals need to be conducted, collecting female samples from captive conditions is feasible as compared to collection of samples from wild population. In order to identify female faecal samples, sexing via PCR becomes an indispensable technique. Also, the determination of sex in natural populations of mammals is essential for understanding population dynamics, management decisions, population structure and habitat use, and behavior and mating systems (Brown et al. 1991a; Gompper et al.1998; Hughes 1998). We are using a PCR-based technique for sex determination in Rhesus Macaque (*Macaca mulatta*) population by DNA isolated from faecal and blood sample. Sex determination via molecular techniques involves PCR amplification of a region of gene(s) specific to X or Y chromosomes by using specific primers.

Once the reproductive cyclicity patterns are recognized for a population immunocontraception will be administered to standardize the effective dose. Post vaccination, anti-PZP antibody titres will be estimated by ELISA to investigate the titres corresponding to induction of contraception.

The study requires to collect faecal and blood sample simultaneous vaccination trials in captivity as well as free ranging setups.

a. Captivity: Proposed animal facility at Wildlife Institute of India, Dehradun, Uttarakhand.

b. Free ranging: Surrounding areas of Wildlife Institute of India, Dehradun, Uttarakhand.

Currently we have started work (sample collection and behavioral study) on free ranging macaque troops in and surrounding area within radius of 2 km of Wildlife Institute of India.

Material & methods

The extraction of the steroids from the faecal matrix represents the initial step before quantification. Organic phase extraction to extract steroids from non-liquid matrices such as dried solids or other organic matter such as faeces and dung. For extraction of steroid, we used ethanol for faecal/ dung samples and di-ethyl ether for serum in order to let the dried extracted steroid completely solubilized because of their limited aqueous solubility. Alternatively, the organic layer can be separated and stored for later use as needed.

Sample collection

Weekly dung samples are collected from four elephant camps Mathigodu, Rampura, Dubarre and Annekadu. In Mathigodu, samples (blood and fresh dung) are collected from 21 elephants for to check for reproductive hormones and their metabolite levels at different stages of estrous cycle. Similarly, 13 elephants were sampled in Rampura camp at Bandipur Tiger Reserve, 20 elephants at Dubarre camp and 3 elephants at Annekadu camp. Dung samples are collected weekly and blood samples biweekly, the separated serum from blood samples is collected in fresh labelled tubes, dung samples for wild elephants are collected as well, all collected samples (serum, dung) are stored at -20^{o}C until further processing. Faecal samples of Rhesus Macaque were obtained from study area around WII. Fresh samples were collected, DNA isolation was done to confirm the species and sex identification by polymerase chain reaction (PCR) using specific primers. Samples were collected using clean tools to avoid contamination of samples through human contact or cross-contamination. Fresh faecal samples were collected in plastic zip lock bag and labelled transported to laboratory and with proper cataloguing frozen at −20^{o}C until use. Once the sexing was confirmed for sample it was further subjected to hormone extraction and assessment.

11.1.1 DNA extraction

DNA from the faeces samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) and manual method, Guanidium thiocynate (GuSCN) method and blood DNA was extracted via QIAGEN DNeasy Blood and Tissue kit. The following procedure is modified from a standard protocol.

Guanidium thiocynate method (GuSCN method)

- Selection of working portion from sample; take small amount of faecal sample which appears to be fresh or shiny on the tail end on a petri plate.
- pipette GuSCN extraction buffer and add on sample (3-4) times (not directly on sample). Wet sample with buffer as long as colour of buffer turns dark (pale yellow for very dry faecal sample).
- Transfer to 1.5 ml microcentrifuge tubes and centrifuge at 14000 rpm for 3 minutes.
- Leave the sample overnight for lysis to get good quality of DNA.
- Add 60 microliter of silica to new tubes and transfer supernatant into it.
- Vortex and then keep it to rotate.
- Centrifuge at 1400 rpm for 90 second.
- Discard supernatant (manually upside down), add 500 microliter GuSCN wash buffer.
- Vortex to mix pellet and buffer completely and centrifuge at 14000 rpm for 90 second. Repeat the washing with 500 microliter ethanol wash buffer, vortex to mix centrifuge at 14000 rpm for 90 second
- Dry the pellet at 56^{o}C till ethanol evaporate (gently hit the tube to check if sample is dry it takes approx. 1 hour (keep tube open)

- Elution; Dissolve pellet in 120 microliter AE buffer (vortex for few second). Keep in 56^{o}C water bath for 1 hour (close tube) Centrifuge at 14000 rpm for 15 minutes
- Take supernatant in new tube Store at 4^{o}C

DNA extraction from kit method protocol

- Weigh 18-220 mg stool in a 2 ml microcentrifuge tube and place on ice.
- Add 800ml inhibit Ex buffer to each stool sample vortex, mix or till it is homogenised.
- Centrifuge sample for 1 min to pellet stool at room temperature at 14000 rpm.
- In a new 105 ml microcentrifuge tube add 25 microliter proteinase K
- Add 600 microliter supernatant (from step 3) in the proteinase K containing tube.
- Add 600 microliter buffer AL and vortex for 15 second thoroughly mix to make homogenous solution.
- Incubate at 70^{o}C for 10 minute.
- Add 600 microliter ethanol (96%-100 %) to lysate and mix by vortexing.
- Apply 600 microliter lysate from (8) to spin column centrifuge at 14000 rpm for 1 min place spin column in new 2 ml collection tube and discard tube containing filtrate. Repeat until all lysate has been loaded.
- Add 500 microliter Buffer AW1 in spin column. centrifuge for 1 min at 14000 rpm. Place spin column in new 2 ml collection tube and discard filtrate.
- Add 500 microliter buffer AW2. Centrifuge for 3 min discard collection tube containing filtrate.
- Place spin column in new 2 ml collection tube and discard old collection tube with filtrate centrifuge for 3 min at 14000 rpm.
- Transfer the spin column into a new labelled 1.5 ml microcentrifuge tube and add 200 microliter buffer ATE directly on membrane incubate for 1 min at room temperature and centrifuge for 1 min to elute DNA.
- Store at 4^{o}C
- DNA extraction from blood sample protocol
- Nonnucleated blood: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge
- tube. Add 50–100 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.
- Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.
- Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard the flow-through and collection tube.
- Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x g. Discard the flow-through and collection tube.

- Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2 and centrifuge for 3 min at 20,000 x g (14,000 rpm). Discard the flow-through and collection tube. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
- Elute the DNA by adding 200 µl Buffer AE to the center of the spin column membrane.
- Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x g.
- Optional: Repeat step 8 for increased DNA yield.

DNA quality check via agarose gel electrophoresis

To check DNA from extracted sample we took 2 microliter extracted DNA, mixed with 1 microliter 6X loading dye and checked on 0.8 % agarose gel. in 1× Tris borate EDTA buffer. The bands were visualized with SYBR Green I (Sigma, St. Louis, MO, USA) (Figure 11.2).

11.1.2 Steroid hormone extraction protocol for Faecal/Dung samples- (Ethanol extraction)

All Samples were dried in Hot air oven set at a temperature 60°C for 3-4 hours. Ensure that the sample is completely dry and powdered to improve extraction recovery. Large particles i.e., grass, stones and inorganic substances were removed, through sieving.

Procedure

- Weigh out 0.2 gm of dried faecal/dung sample into a tube.
- Add 1 mL of ethanol (80%) for every 0.1 gm of sample (0.1 gm fecal/dung /mL) and seal.
- Shake vigorously for at least 30 minutes.
- Centrifuge sample at 5,000 rpm for 15 minutes at 4°C.
- Reserve supernatant in a clean tube.
- Transfer a measured volume of supernatant from step 4 (Evaporation Vol.) into a clean tube and evaporate with the help of airflow.
- Samples containing low levels of analyte can be concentrated by drying down extract and reconstitute in a reduced volume of Assay Buffer. Vortex well and allow to rest 5

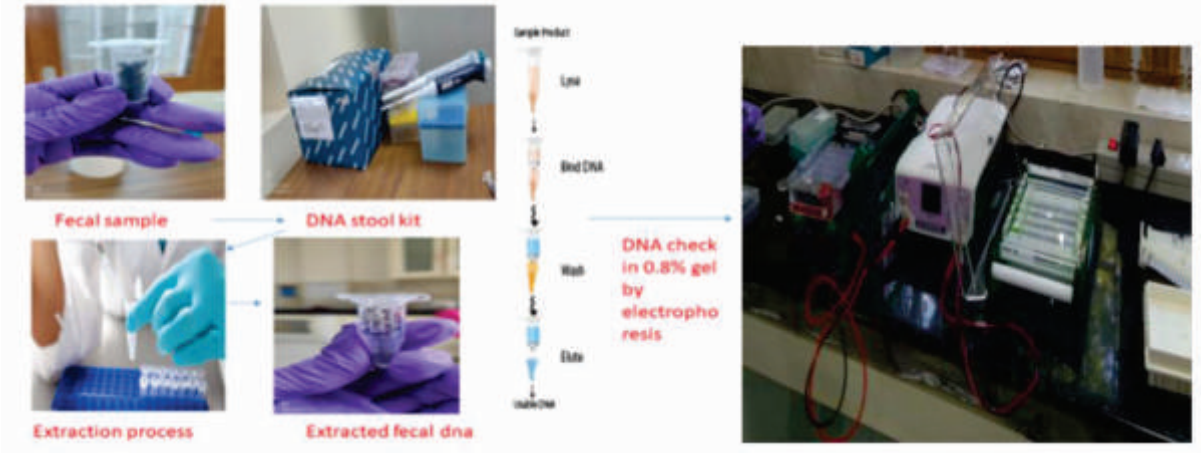


Figure 11.1 : *Stepwise process of DNA extraction and quality check.*

minutes at room temperature. Vortex and rest for 5 minutes twice more to ensure complete steroid solubility.

- For immunoassays, the ethanol content in the assay typically must be 5%. This will require additional dilution into Assay Buffer.

11.1.3 Steroid Extraction protocol for Serum sample- (Diethyl ether extraction)

- Add diethyl ether to serum sample at 5:1 (v/v) ether: sample ratio.
- Mix solutions by vortex for 2 minutes. Allow ether layer to separate for 5 minutes.
- Freeze sample in ethanol bath and pipet out the ether solution from the top of the sample into a clean tube. Repeat step 1-3 for maximum extraction efficiency, combining top layer of ether solutions.
- Dry pooled ether sample down in airflow for 2-3 hrs. If sample need to be stored it should be kept at -20^{o}C.
- Reconstitute the sample at room temperature in the Assay Buffer.

11.1.4 Hormone analysis

We are using Commercial Hormone ELISA kit DetectX from Arbor Assay for our study from extracted faecal, dung and serum samples of selected species. The following procedure is modified from a standard protocol.

Reagent Preparation

Allow the kit to come to room temperature for 30 minutes. Ensure that all sample have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer (Dilute Assay Buffer Concentration 1:5 with deionized water).

Wash Buffer (Dilute Wash Buffer Concentration 1:20 with deionized water).

Standard Preparation- The concentration of steroid take standard 1 to standard 7 will be 10,000, 4000, 1600, 640, 256, 102.4 and 40.96 pg/ml.

Assay Protocol

- Make plate layout sheet to aid in proper sample and standard identification.
- Pipet 50 µl of samples or standards into wells in the plate.
- Pipet 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
- Pipet 50 µl of Assay Buffer into the maximum binding (B0 or Zeroo standard) wells.
- Add 25 µl of the DetectX Progesterone conjugate to each well.
- Add 25 µl of the DetectX Progesterone Antibody to each well, except the NSB wells.
- Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signal bound will be approximately 20% lower.

- Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
- Add 100 µl of the TMB Substrate to each well.
- Incubate the plate at room temperature for 30 minutes without shaking.
- Add 50 µl of the stop solution to each well, using a repeater pipet.
- Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
- Calculate the Progesterone concentration for each sample by using a 4PLC software.

Results

Sex identification using PCR- We tested all primer sets with isolated Faecal & blood DNA samples. Sex identification was done by using previously collected 4 blood samples with PCR reaction by using mammalian housekeeping primer RBG and sexing primer ZFY. 10µl of PCR product was mixed with 2 microliter 6X loading dye and loaded into the wells and electrophoresed on a 2 % agarose gel in 1× Tris borate EDTA buffer, a 100-bp ladder marker (Invitrogen) was loaded in a separate well to identify product size. The bands were visualized with SYBR Green I (Sigma, St. Louis, MO, USA) under Gel documentation system. (Figure 11.2). 2 samples out of 24 tested samples we were able to identify sex. D2F20 was detected female and A4D19 as male via ZFY sexing primer, we took previously identified blood DNA samples (one male and one female sample) as positive control.

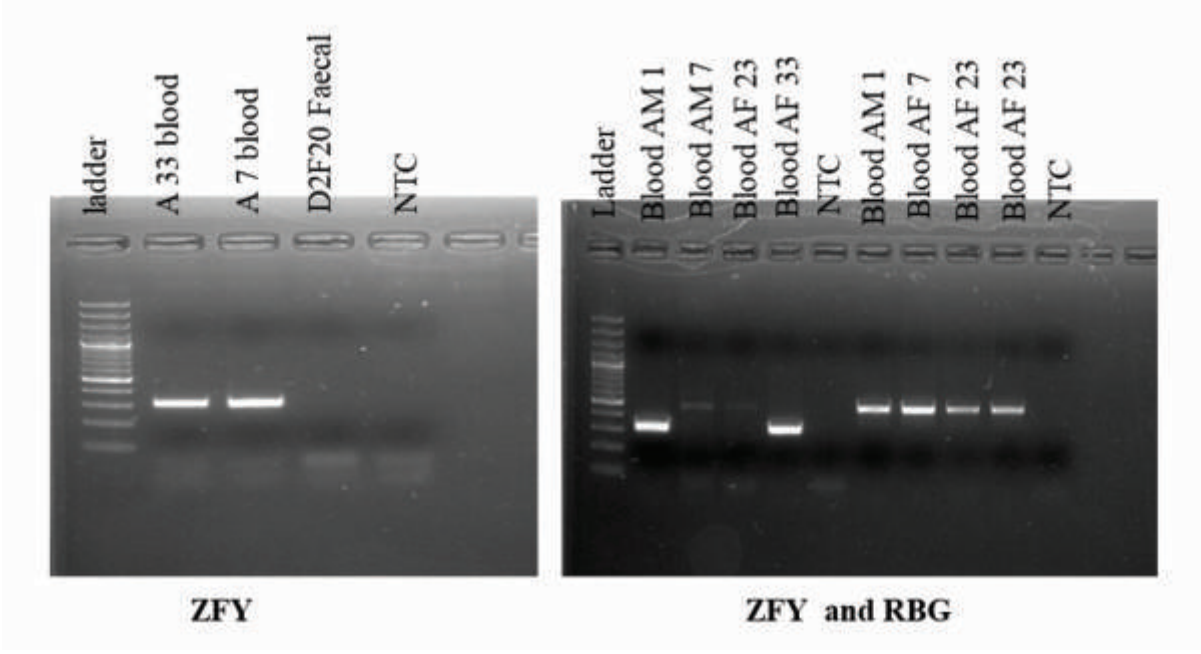


Figure 11.2 : *Amplified PCR products with marked size using Faecal DNA and blood DNA analyzed by agarose gel (2% agarose) electrophoresis and bands (mark with sample ID) are visualized using SYBR Green I under Gel documentation system (ZFY-310bp & RBG-480bp).*

Reproductive Hormone estimation from faecal/dung & serum samples

Initial data suggests the average progesterone levels in rhesus macaque female fecal samples collected during May-August (non- breeding season) were 9.6 ng/ml, whereas the average levels for samples collected during October- April (breeding and parturition seasons) were 13.14 ng/ml. Elevated level of progesterone is indicator of transition from follicular phase to luteal phase or successful conception. 17β-estradiol levels were estimated in serum samples, with the dilution used in assay the detection was below LOD for most of the female elephant serum samples and the detection range was 16.86pg/ml to 161.90 pg/ml. The reason behind no detection could be the sample dilution used for assay or the estrous cycle stage of females for which weekly samples are required. For rhesus macaques the range was 153pg/ml to 513pg/ml for 17β-estradiol. Female rhesus macaques were captured during December 2020-March 2021 and blood samples were collected for serum isolation to be used for reproductive hormone analysis. 80% of the female rhesus macaques captured were found to be pregnant as confirmed by ultrasonography. Faecal Estrogen Metabolites (FEM) and Faecal Progesterone Metabolites (FPM) are under analyses using faecal/dung samples.

11.2 Detection of pregnancy using Ultrasonography

Free-range adult females of rhesus macaque groups in Wildlife Institute of India were captured from different location of institute (December 2020 to March 2021) to monitor the fetus health and pregnancy diagnosis. The number of adult females captured, pregnancy diagnosis and pregnancy percentage are presented in table No. 11.1.

Materials & methods

Ultrasound examination was carried out to detect the pregnancy and to monitor the embryonic and fetal development in rhesus macaque troops. The fetus was evaluated for viability, heart rate and pregnancy stages. Transabdominal ultrasound examination under anaesthesia was performed in adult female macaque monthly from December 2020 to March 2021. The hair at abdominal area were clipped and cleanly prepared for the examination. The ultrasound examination was performed in dorsal recumbency. Ultrasound gel (Smartcare SC-GE-250) was applied to abdomen.

Table 11.1 : Month wise pregnancy status of captured free-ranging adult Rhesus Macaque females.

Month of capture	No of total captures	No of adult females	No. of pregnant female(s) confirmed	Percentage (%) pregnant female
December 2020	1	4	2	50.00%
January 2021	2	3	3	100.00%
February 2021	1	2	2	100.00%
March 2021	2	4	3	75.00%
TOTAL	6	13	10	76.92%

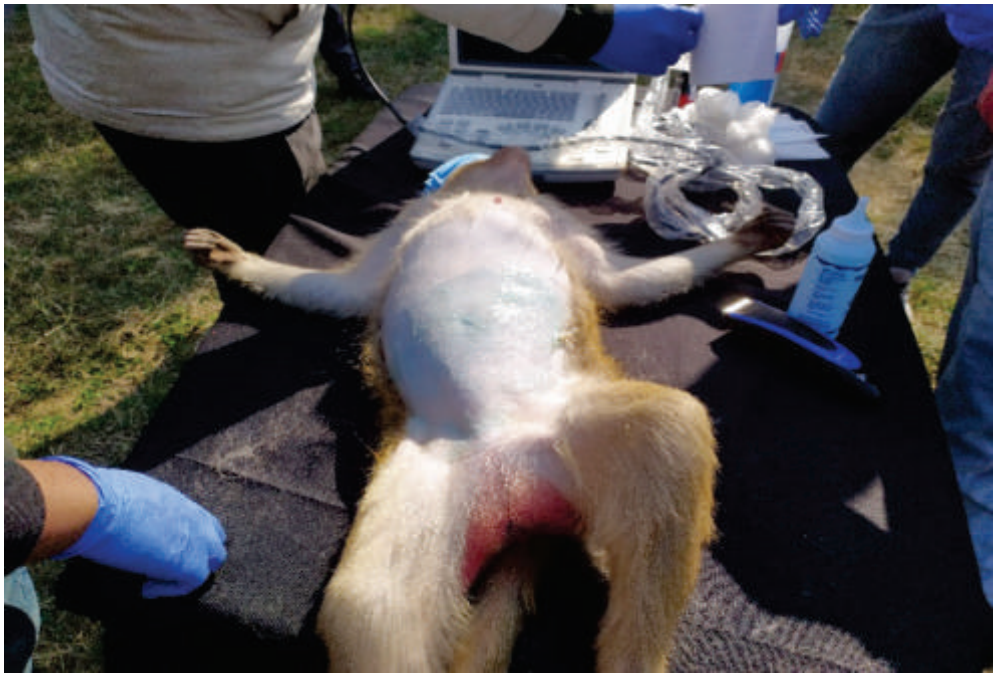


Figure 11.3 : *The uterus of animal was screened for presence of fetus by using 65C15EAV transducer (MIndray Dp 30 VET) in longitudinal and transverse position on the lower abdominal. A frequency of 5-8.5 MHz and depth 2.5 – 6 cm were used. The normal uterus of female was visualised by ultrasound examination.*

Figure 11.4 : *To measure the gestational sac, fetal head and heart, the transducer was moved across the female abdomen until the fetus or uterus was visualised. The transducer was rotated and moved to give the clear visualisation of foetus head and heart. Featal head diameter and heart rate was measured to evaluate the normal morphology. The thoracic area of foetus for heart rate, spine, stomach and other structures were visualised as per the echogenicity of ultrasound image.*

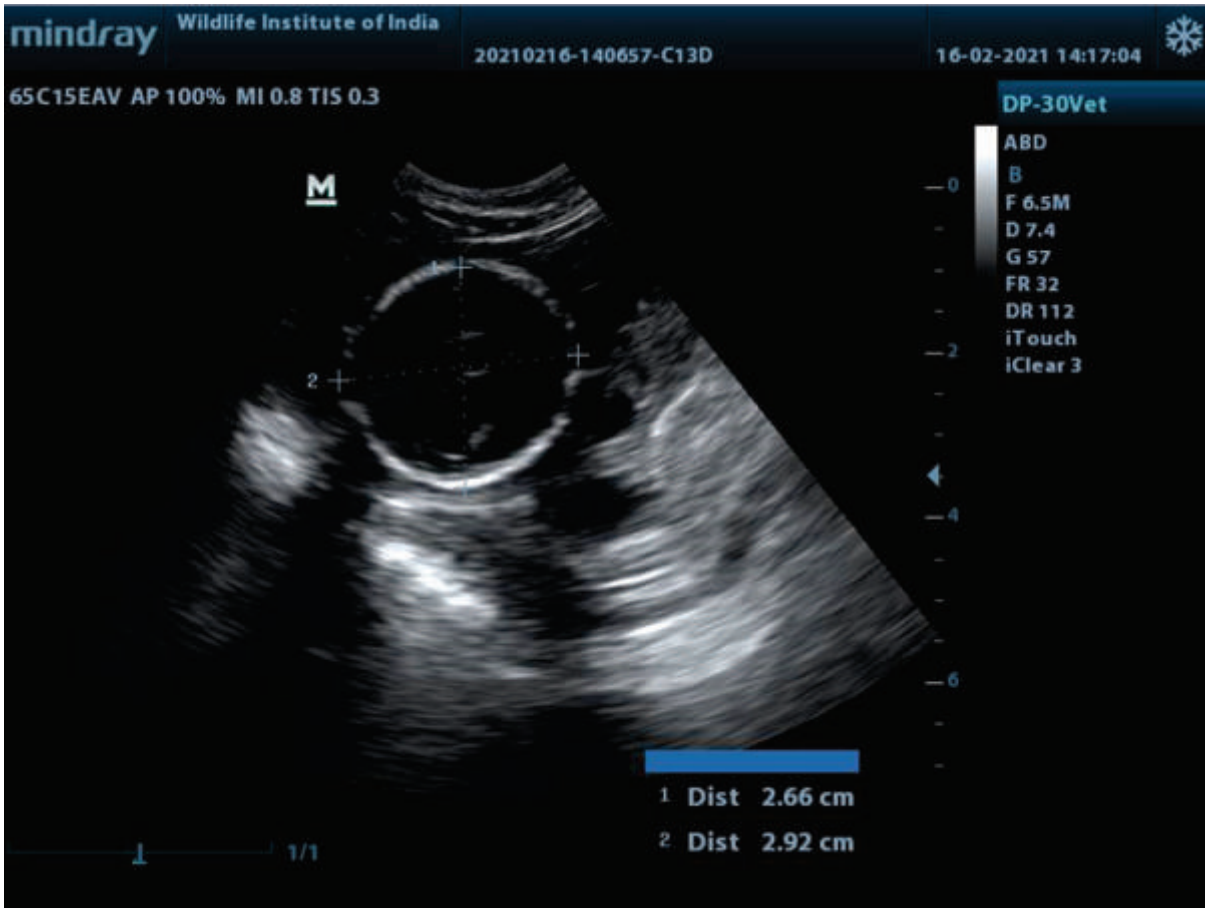


Figure 11.5 : *Foetal head diameter*

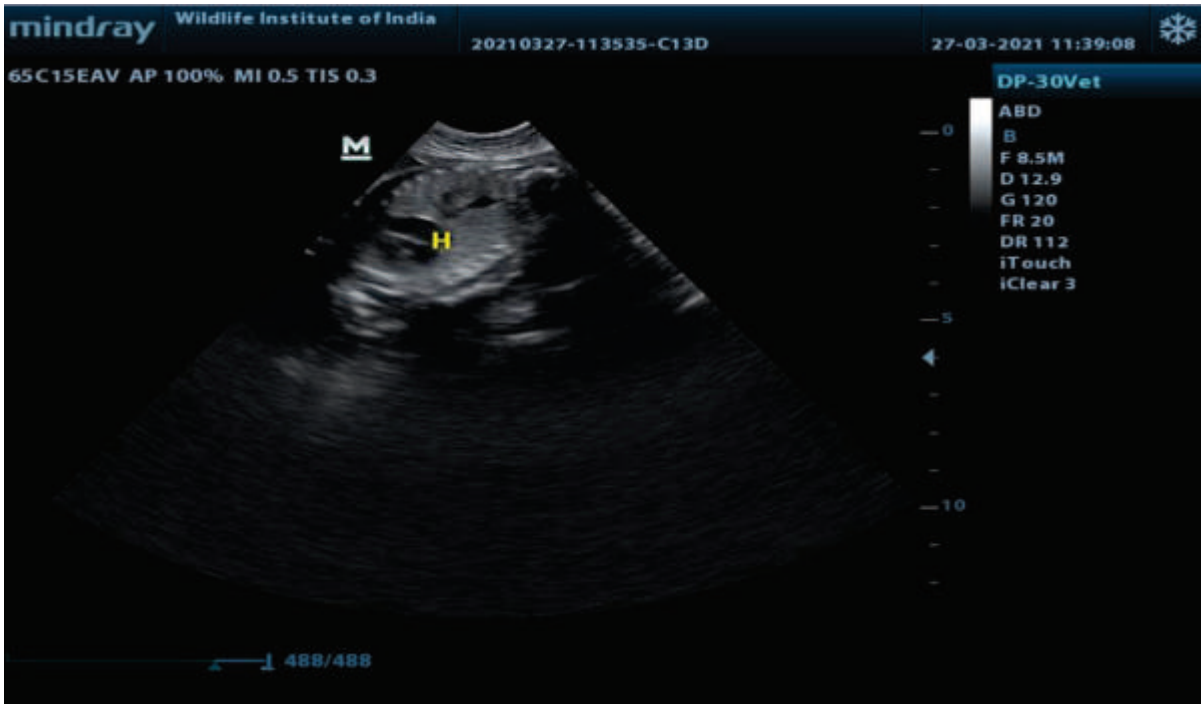


Figure 11.6 : *Foetal heart (H)*

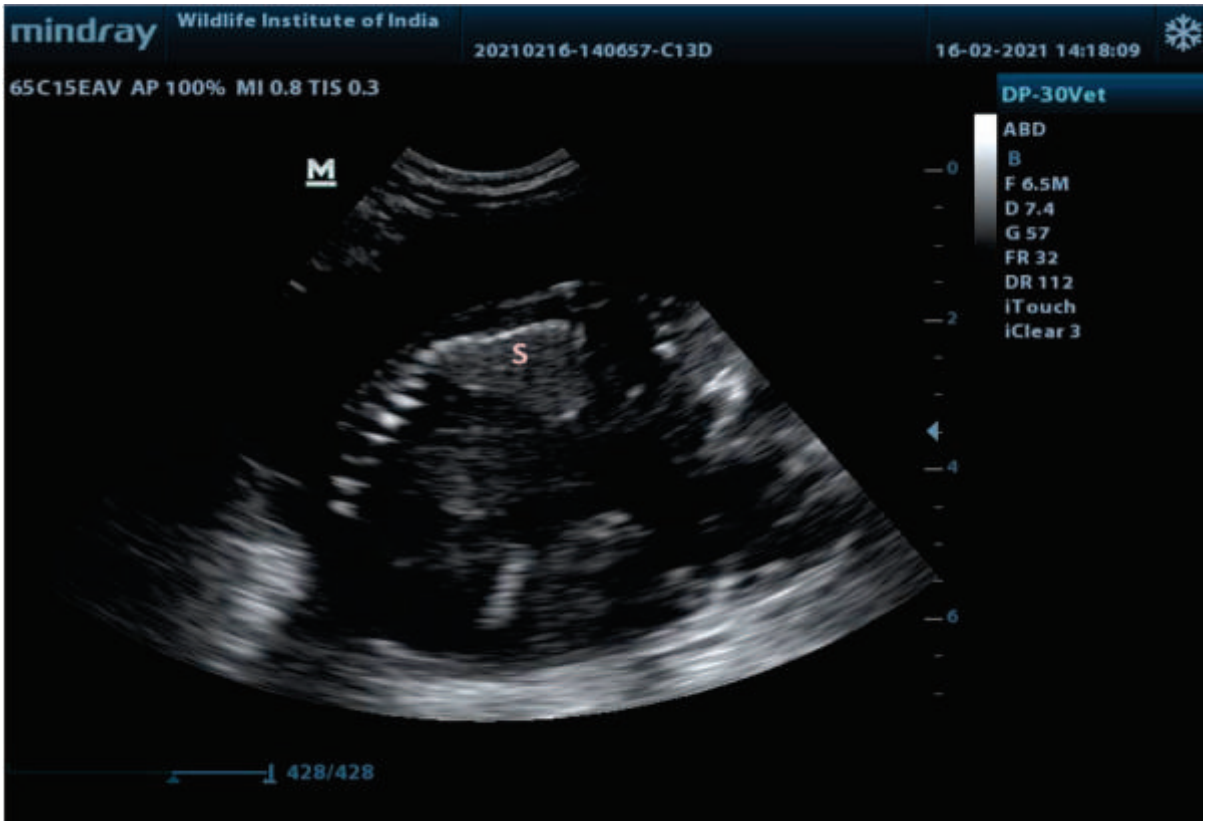


Figure 11.7 : *Foetal spine (S)*

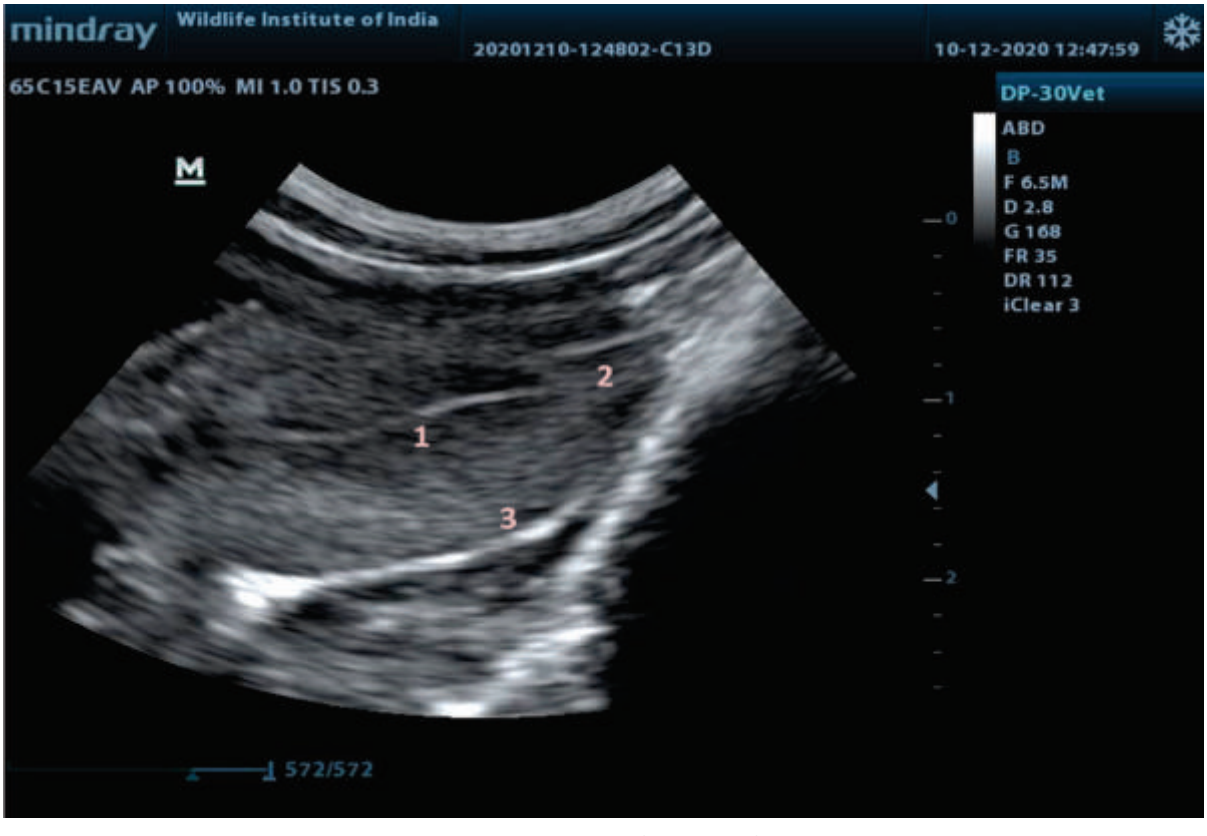


Figure 11.8 : *Ultrasound image of uterus of rhesus macaque.*
1. The lumen (thin stripe) 2. Endometrium (dark area) 3. Myometrium (dark grey area)

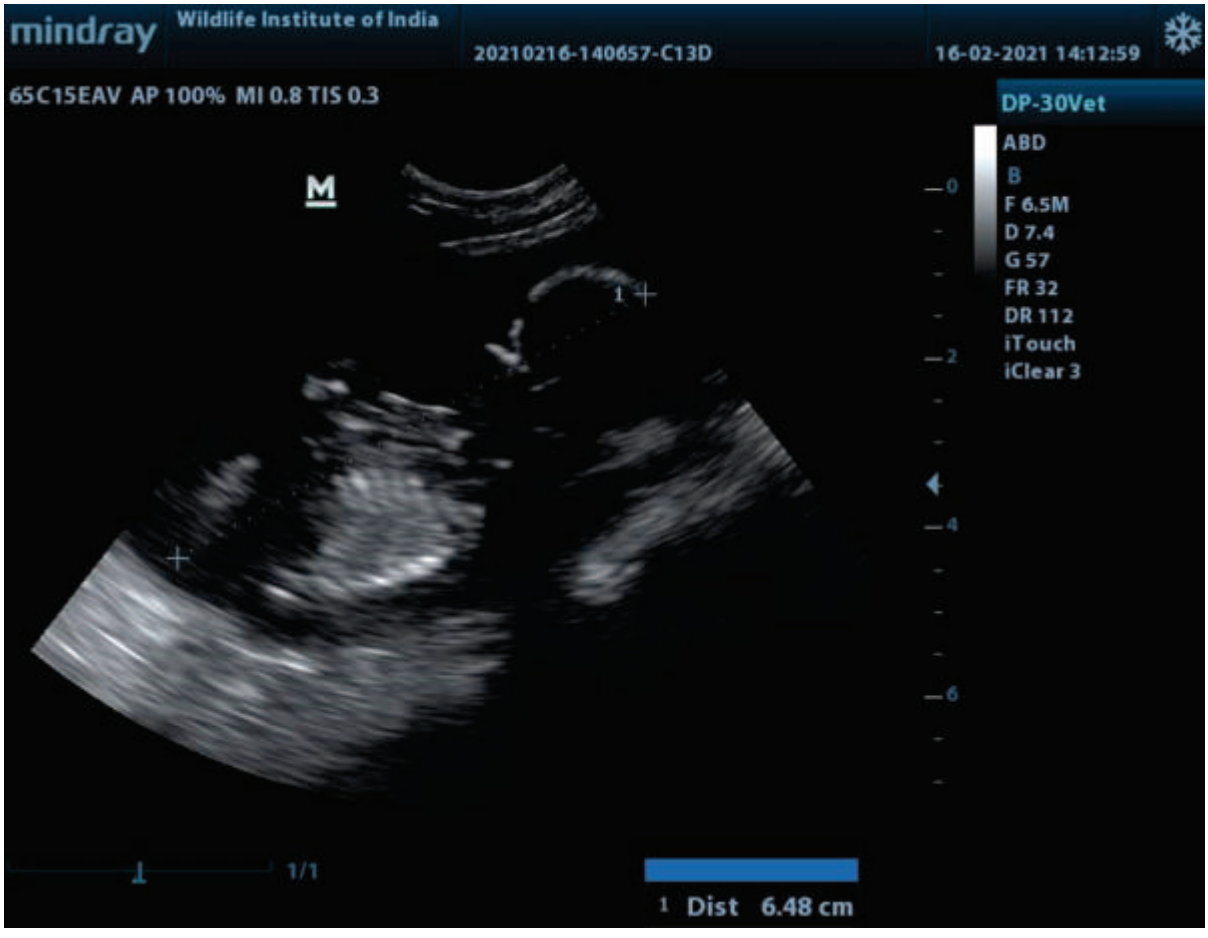


Figure 11.9 : *Crown rump length of rhesus macaque foetus*

Results

Pregnancy diagnosis by ultrasound examination in 13 adult female rhesus macaques was performed from December 2020 to March 2021 (table 11.1). Confirmation of pregnancy in females was based on visualisation of gestation sac (first trimester) and presence of fetus. The growth of fetus was monitored month wise in different females of troops captured based on fetal heart rate, fetal head diameter and crown rump length. Pregnancy percentage (76.92%) was observed in females until March 2021. Rapid and strong fetal heart rate (125-145 bpm) was observed in pregnant females indicating healthy pregnancy.

11.3 Reproductive Control Studies

11.3.1 Immunocontraception

Immunocontraception utilizes the ability of immune system to generate antibodies to achieve fertility control. Reproductive peptide hormones, proteins/ peptides are prominently expressed in gametes (spermatozoa or oocyte) and other proteins crucial for the process of gamete fusion when injected into body along with appropriate adjuvant are capable of mounting an immune response. As a result, the body produces antibodies against them, subsequently leading to interruption in the function of target protein. Fertility controls can be

achieved for a duration of 1-4 years or longer.

ZP (Zona Pellucida) based immunocontraception has been tested successfully in captive and free ranging wildlife species. Antibodies against ZPGs (Zona Pellucida glycoproteins) block spermatozoa and oocyte binding or can interfere with oocyte maturation within the follicle. ZP based vaccination induce infertility via blocking fertilization for the stipulated time, the already fertilized egg remains unharmed. Even if the pregnant animal receives vaccination there will be no adverse impact on foetus. Few adverse reactions have been reported but they are most like to be attributed to impure or crude preparation of the antigen. Approval of contraceptive agents for use in animals especially the wildlife species takes into consideration the ecological and economical aspects as well. The prerequisites for a successful immunocontraceptive vaccine are, it must be safe with minimal side effects on the animal and must be able confer infertility for longer period of time. Anti-fertility agents which are not persistent and non-toxic to ecosystems are encouraged. ZP based vaccinations are studied extensively as compared to other available options. The field studies performed has provided descriptive data and insights into usage of ZP based vaccines as immunocontraceptives. ZP based vaccines full fill the criteria of effective immunocontraceptive vaccine design, ZP is an important component of gamete development fertilization process, it is capable of mounting an immune response when it is injected in the animal and the antibody response generated is long lasting and causes suppression of fertility. Moreover, there is no harmful impact on the environment and on other non-target species, since antibodies generated are just a miniscule fraction of various other proteins in the sera, also biomagnification is not applicable. The route of administration is either subcutaneous or intramuscular so there is no interaction with soil, water and air; non-target species have no chances of getting exposure to vaccine. Quantity of antigen is very small, at nanograms and micrograms scale per vaccination per animal. No adverse impact on social and reproductive behaviour have been observed. The general as well as reproductive physiology of animals is not altered; the contraception is reversible. As soon as the titres drop the animal regain the reproductive vigour.

11.3.2 Generation of Polyclonal antibody in rats

Immunization of rats for generation of polyclonal and Monoclonal antibodies is an indispensable tool in immunological studies. Finding extensive application in immunohistochemistry, western blotting and ELISA, antibodies specific to target antigens are extremely useful. While working on novel antigens, commercial antibodies may not be specific or unavailable. To overcome this issue in-house generation of polyclonal/monoclonal antibodies can be done.

In addition, assessment of efficacy and safety of Zona pellucida based immunocontraceptive can be performed. The antibody titer estimated post vaccination can be correlated to the fertility outcome. Also, mating behavior of subject animals and impact on primary and secondary reproductive organs can be observed to establish the safety of immunocontraceptive.

Table 11.2 : Experiment Plan for Vaccine trial in laboratory rodents.

Procedure	Protocol Day	Group 1/Control	Group 2	Group 3
Control serum collection	Day 0	Yes	Yes	Yes
Primary Immunization	Day 1	CFA+PBS	CFA+PZP	CFA+PZP
1st Booster	Day 21	IFA+PBS	IFA+PZP	IFA+PZP
2nd Booster	Day 42	IFA+PBS	IFA+PBS	IFA+PZP
Test Bleed	Day 50	Yes	Yes	Yes
ELISA	Day 50-55	Yes	Yes	Yes
Decision-Reproductive study	Day 55 onwards	Allow all groups to reproduce		

11.3.3 Experimental Plan

Female Sprague-Dawley/ Wistar rats, 6-8 weeks of age will be divided into 3 groups- control and PZP immunization (single booster dose and two booster doses) (N=5 each). Animals will be maintained in 12-hour L/D cycle with ad libitum access to food and water. Animals in immunization group will be primed using CFA (Complete Freund's Adjuvant) and boosters will be administered using IFA (Incomplete Freund's Adjuvant). Control group will be administered adjuvants only. As per the plan mentioned below the animals will be assessed for immune response via production of anti-PZP antibodies.

11.3.4 Alternative strategies of Reproductive control

Different approaches have been tested to control fertility in wildlife species, some of the methods are based on chemicals that interfere with ovarian function and others involve use of synthetic reproductive hormones or analogues which impact ovulation or implantation in female animals or spermatogenesis in male animals. The outcomes on fertility rates could be permanent or temporary depending upon the strategy applied. Occupational chemicals like VCD (4-vinylcyclohexene diepoxide), a by-product of insecticides and rubber synthesis has been tested both in vitro and in vivo for its potential toxicity to oocytes. VCD has been used to develop experimental menopause model in mice, rats and non-human primates. It is reported to deplete the ovarian oocyte pool as observed in cynomolgous macaques, dogs, rats and mice. We have designed a study to test the efficacy of VCD as potential reproductive control agent. As mentioned above the overall assessment of reproductive control agent is done for its safety (side- effects on biology of animal and non- target species) and feasibility (administration, dosage, impact on environment) in addition to its efficacy. This study includes the assessment of toxicity of VCD on various organs, effective dose, route of administration and other parameters related to safety and feasibility of application.

Alternatives to PZP based immunocontraception need to be investigated as well, since it might not be the antigen of choice for some of the study species like wild pig due to porcine origin of ZP. Immunocontraception is one of the long- term approaches we intend to test and implement at large scale. Prior to application in field/ large scale, we need to test for safety, efficacy and feasibility at designated trial centres/ captive facilities (research purpose). In case of wild pigs, we will test GonaCon at captive facilities within premises of Wildlife Institute of India, Dehradun, India. 20 Wild/feral pigs will be captured quarantined and further used for trials.

Chapter 12

POPULATION MANAGEMENT SCENARIOS

Qamar Qureshi, Uddalak Tathagato Bindhani, Mariyam Nasir, Souritra Sharma, Chandrapratap Singh Chandel, Sanath Krishna Muliya, Lallianpuii Kawlni, Vishnupriya Kolipakam.

Human-wildlife conflicts are multifaceted and cannot be easily explained or resolved. To gain insights into the intensity, complexity, and potential approaches to address these conflicts, we looked into population management scenarios. It is only part of solution. Other aspects which include socio-political dynamics need separate study. Income levels can also impact human-wildlife conflicts. Communities experiencing poverty may have a higher tolerance for losses caused by wildlife due to their reliance on natural resources for subsistence. In contrast, individuals with higher incomes may have more resources to invest in protective measures and may be less tolerant of wildlife impacts. Land ownership is another significant factor. Conflicts often arise when wildlife damage crops, livestock, or property. The degree of control and responsibility over the land can influence the perceptions and responses of landowners towards wildlife. The specific species involved in the conflicts can also influence the nature and intensity of the conflicts. Some species may cause more severe damages or have cultural or religious significance, leading to heightened tensions and conflicts.Overall, these factors, such as education, income levels, land ownership, and species characteristics, can interact and contribute to the complexity of human-wildlife conflicts. Understanding these correlations can inform the development of targeted strategies and interventions to address and mitigate conflicts more effectively (Liu et al., 2011; Suryawanshi et al., 2013; Zimmermann, 2014).

A framework for population control is divided into urban and rural conflict area, having gradient of conflict and area which are at interface with Protected Area or Reserve Forest Area where population of species in conflict occupy natural and human habitat.

Method

Population performance in different scenarios was performed in Vortex software package (version 10) (Lacy 1993).

The initial population size (N_0) was based on empirical data from study area or representative published literature, respective to the species. The carrying capacity was set as twice the initial population size. Hundred iterations were run for each model and the time period of the simulation was set for hundred years. Extinction for the species was defined when the simulation predicted the survival of only one of the sexes. The standard deviations were set at 10% of the corresponding mortality rates at that age. The percentage of males at birth was maintained at 50%.

Simulations were run at varying levels of non-breeding females based on the assumption of the percentage females targeted with management interventions (immunocontraceptives etc.) until an extinction probability was achieved.

12.1 Rhesus macaque (*Macaca mulatta*)

We examined two population scenarios; one focused on the troupe and the other on the overall population within the study area. The overall population density estimates of Rhesus macaques in the 16 sq. km. study area (Chandrabani, Dehradun) was ~143 individuals/km^2. The total sampling effort was 604.5 km (2020-2023).

For the population level analysis, the initial population size (N_0) was set at 2288 with a carrying capacity of 4576 ($2N_0$). Details for the reproductive system of the Rhesus macaque were as per published literature on the species (Rawlins and Kessler 1986; Fooden 2000; Cawthon Lang 2005). The maximum age of female and male reproduction was 20 and 15 respectively. The maximum lifespan of the species was set at 25. Age of first offspring for females was 3 and for males was 8. The number of broods and progeny for a year was one while the infants were made dependant on their mothers for a period of one year. The percentage of males at birth was maintained at 50%. Mortality rates for the study were adapted from data provided for survivorship by Meikley and Vessey (1988). The initial scenario was simulated as reported in natural conditions whereby 70% of the total adult females are found breeding (Fooden 2000). Population management intervention scenarios were set at 18% and 4% of the total breeding adult females respectively.

The population level scenarios for the natural conditions (70% breeding ♀) at N_0=2288 showed an intrinsic rate of increase (r) for the present population at 0.0841. When 18% of the adult females are allowed to breed, r= -0.064 with an extinction probability of 0.52 and a population half-life ($t_{1/2}$) of ~11 years. When only 4% of the adult breeding females are allowed to breed, r

drops to -0.176 with an extinction probability of one and a $t_{1/2}$ of 4 years.

In case of a troop for N_0=43, natural conditions show an intrinsic rate of increase at 0.091. When 18% of the troop's adult females breed, extinction probability of 1.00 is achieved with r=-0.066 and $t_{1/2}$ of 11 years. When only 4% of the adult breeding females are allowed to breed, r drops to -0.164 with an extinction probability of 1.00 and a $t_{1/2}$ of 4 years.

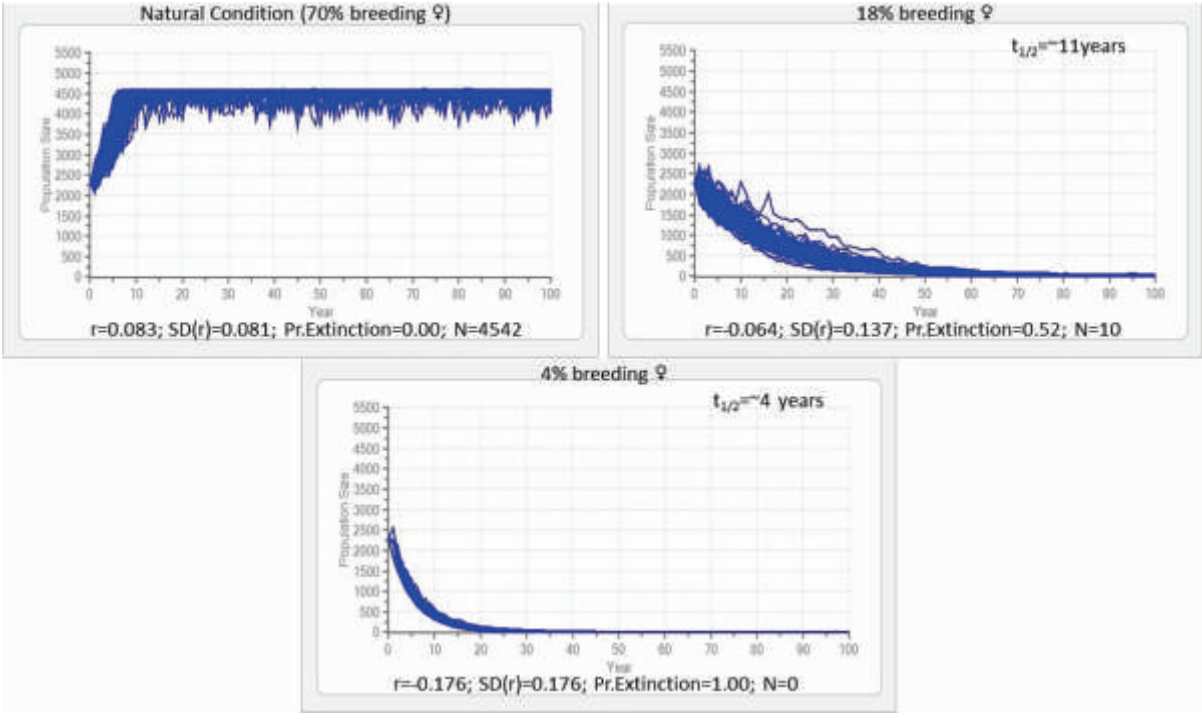


Figure 12.1 : *Population level scenarios for management interventions in Rhesus macaques.*

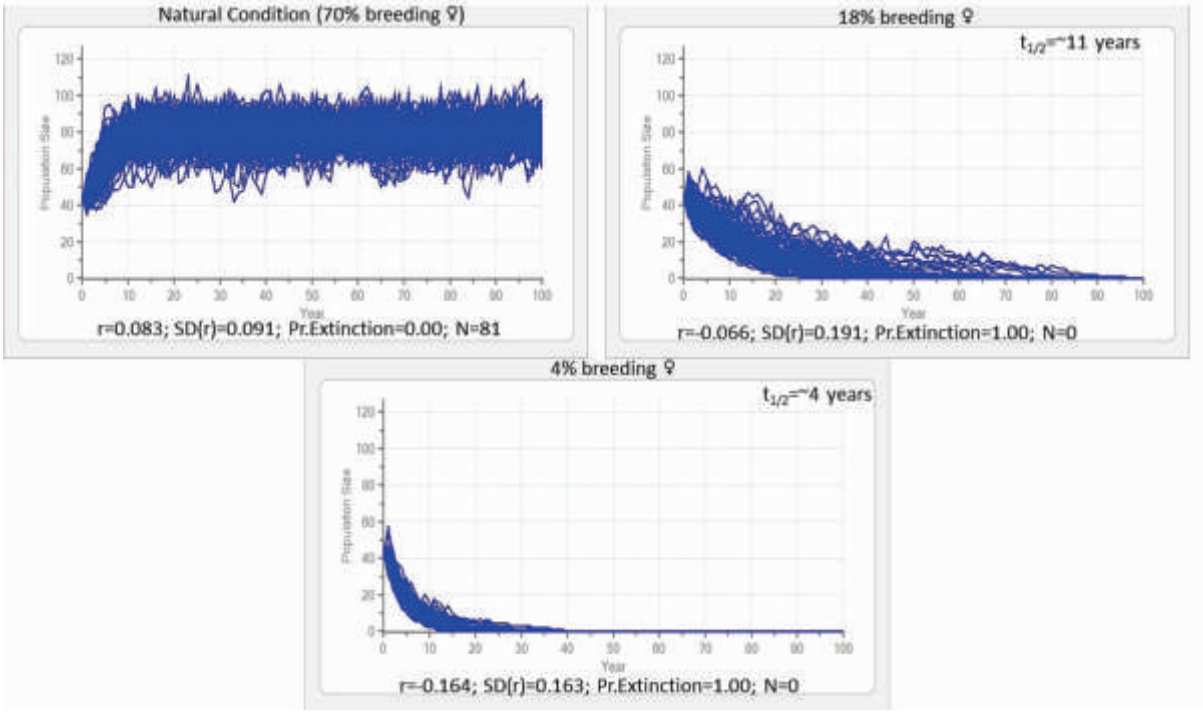


Figure 12.2 : *Troop level scenarios for management interventions in Rhesus macaques.*

Population control

The population management scenario is addressed at two levels

1) Rural and urban area

In high conflict areas, capture most of the macaque and move them to rescue centre. All adult monkeys should be sterilised using surgical procedure, the sub adults should be kept separately and their reproductive control should be done at reaching adulthood. In moderate and low conflict area most of the monkeys (100%) should be sterilised using surgical procedure and released in same area. The procedure should be continued for 5 years till entire population is captured and sterilized. Further course of action need to be decided at this juncture for continuation of the program.

2) Rural or urban area with forest interface

In interface area which is either Protected Area or large Reserve forest 100% of adult monkeys should be sterilised using surgical procedure and then the sub adults of that population in following year when they reach adulthood 80% should be sterilised surgically and 10 % adult should be treated with immuno-contraception like PZP for at least 5 years. The population should be monitored and adaptive process should be followed to decide what percentage will be reproductively controlled and the method of contraception surgical or immune-contraceptive and what proportion in each treatment need to be decided as per situation.

Population monitoring: Population and its characteristics need to be regularly monitored using line transect and facial recognition based Mark-Recapture method involving people in affected area.

12.2 Elephant (*Elephas maximus*)

The initial population size (N_0) was calculated as per the mean herd size of our targeted 3 study herds. N_0 was set at 40 with the carrying capacity at 80 for reported naturally occurring herd sizes (Sukumar, 2003). We used the published reproductive details and mortality rates of the Asian elephant from South India (Sukumar, 2003). The maximum age of female and male reproduction was 60 years both. The maximum lifespan of the species was set at 60. Age of first offspring for females was 15 and for males was 17. The number of broods and progeny for a year was 1 while the inter-calving interval were 5 years. The initial scenario was simulated as per natural conditions were 30% of the females are usually breeding (Sukumar, 2003). Simulations were run at varying levels of non-breeding females based on the assumption of the percentage females targeted with the immunocontraceptives until an extinction probability was achieved. We have run the simulations at 30% of the females reproducing in the natural population. Further we run the simulations at 15% and 3% females breeding in a herd.

The scenarios for the natural conditions in our herds case (30% breeding ♀) at N_0=40 showed an intrinsic rate of increase (r) for the present population at 0.051. When 15% of the females from

the population were allowed to breed, the intrinsic rate of increase decline to, r= 0.027. When 3% of the adult breeding females are allowed to breed, r drops to -0.026 with an extinction probability of 0.96 and a $t_{1/2}$ of 26 years.

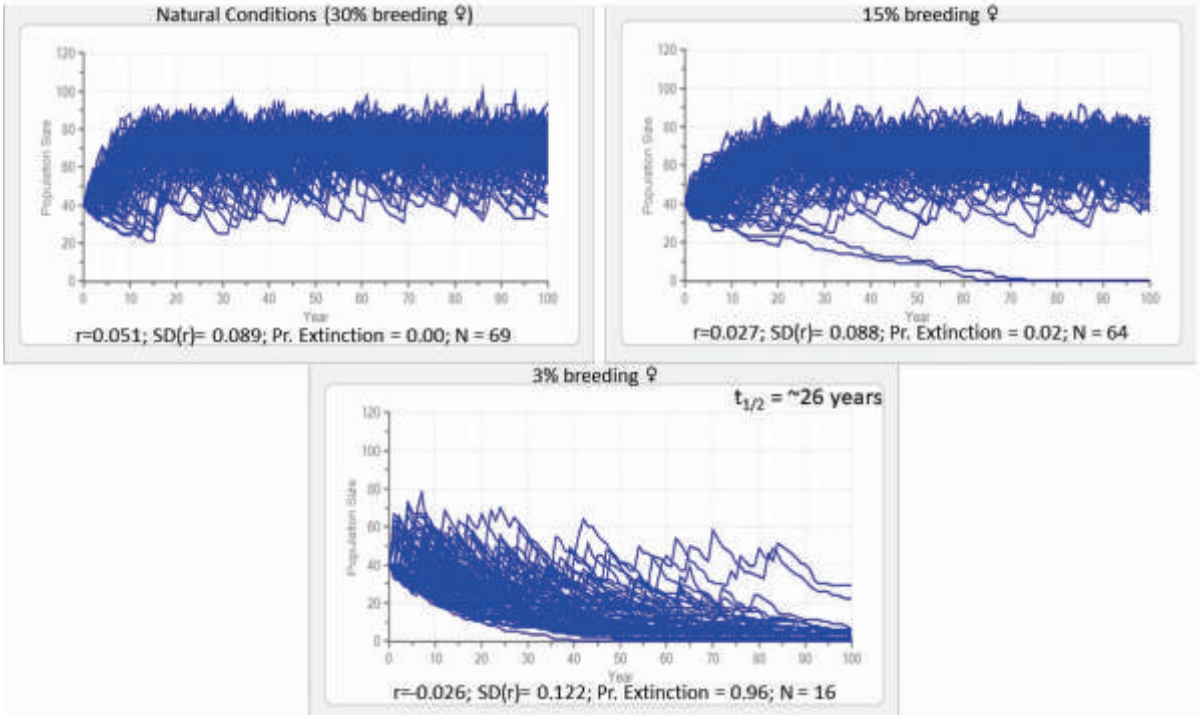


Figure 12.3 : *Herd level scenarios for management interventions in Asian elephants.*

Population control

Immuno-contraceptive like PZP which is found safe in African elephants should be used initially for 5 years and then decision should be taken based on local condition to continue the program depending on population monitoring data.

Population monitoring

Herds need to be monitored physically and using camera trap based distance method to check demographic parameters which will assist in making population management decisions.

12.3 Wild Pig (*Sus scrofa*)

We set the initial population of Wild pigs as 620 per 100 sq km based on the density updates of Wild pigs in Panna Tiger Reserve (All India Tiger Report, 2018). The reproductive age sex and class of wild pigs were set where the maximum reproductive age of females was considered to be 8 years whereas that of males was 10 years with an average lifespan of 12 years. Both females and males reach reproductive maturity within months and have their first brood by the first year. The mortality rate of males before age one is 15% whereas there after it is 4%. Similarly in females, the mortality rate before age one is 7% whereas and there after it is 8% (Adkins, R. N., & Harveson, L. A.,2007). The Wild Pig's mean annual growth rate was considered

to be around 30-40% based on a study in Texas (Mellish et. al, 2014). The natural conditions were set as 50% breeding of all adult females. Population management intervention scenarios were set at 10% and 5% of the total breeding adult females respectively.

The reproductive rates were modified so that we can see in how many years that population will get extinct or decline when the intrinsic rate of growth rate is negative. Simulation models were run based on natural conditions, where 50% of females are breeding with an initial population size (N_0) = 620 showing an r of 0.377 with an extinction probability of 0. When only 10% of the females are breeding, r drops to -0.099, $t_{1/2}$ of 7 years with an extinction probability of 0.99. When 5% of the females are breeding, r=-0.195, $t_{1/2}$ of 4 years with an extinction probability of one.

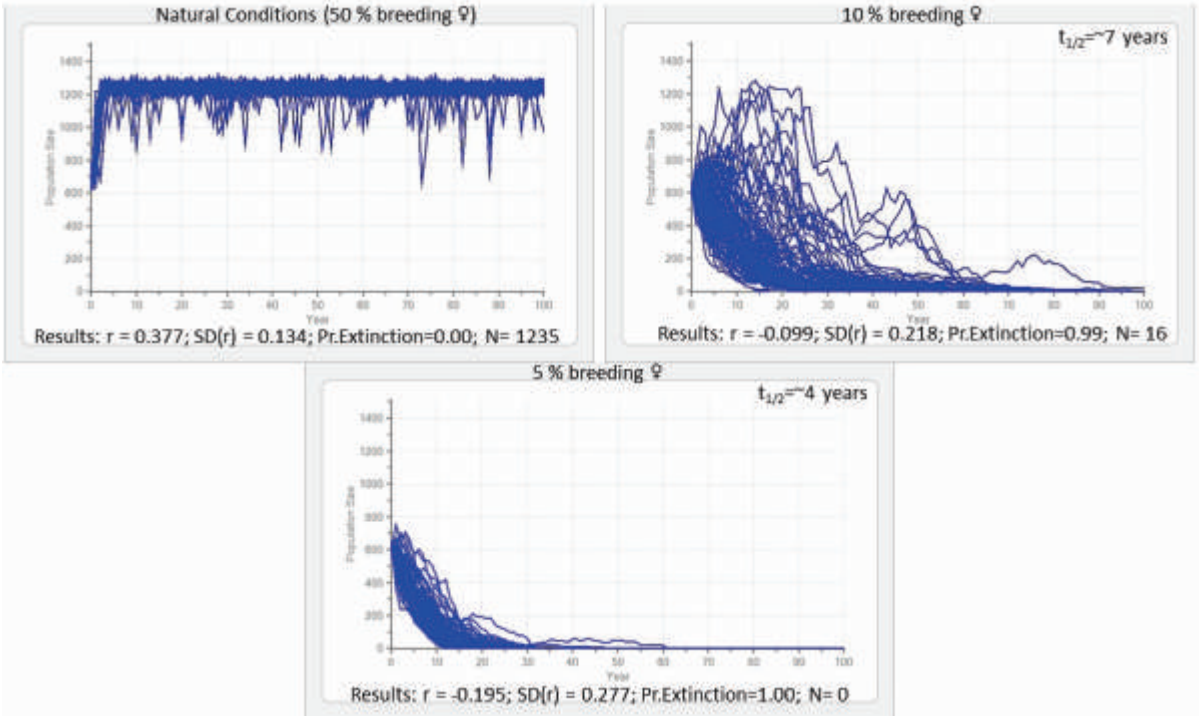


Figure 12.4 : *Population level scenarios for management interventions in Wild pig.*

Population control

There is possibility of chemical control but these procedures are costly and logistically challenging. It is suggested to do mass capture operation using boar buster cages. In some states wild pig was declared vermin and hunted but limited success temporarily was achieved. The captured pigs either used for augmentation in protected areas with low ungulate densities or utilised for consumption depending on cultural and logistics constraints.

Population monitoring

Population where operation is carried out need to be monitored using camera trap based distance sampling for demographic response to interventions used.

12.4 Nilgai (*Boselaphus tragocamelus*)

Demographic parameters of nilgai were borrowed from south Texas population (Sheffield et al., 1983). Age of sexual maturity in females - 3 years, males - 4 years, maximum life span - 15 years, number of broods per year - one, number of progenies per brood is one, sex ratio at birth-50%, maximum age of female reproduction - 10 years, male - 10 years. According to Sheffield et al (1983) in a sample of die-off specimens, the percentage of female lactating or pregnant were 72% and the twinning rate was found to be 50%. We adjusted the percentage of adult females breeding in natural condition by 18% to account for twinning hence the percentage of females breeding in natural condition will be 90%. The initial population size was estimated from the nilgai density at Panna Tiger Reserve, AITE (2018) for 100 Km^2 area which is 1200 individuals. The carrying capacity was kept double of this initial population size 2400. The first simulation was run at natural condition where 90% of females were breeding. The next simulation was run at 18 and 9%. Adult females breeding.

Under natural conditions where 90% of females breed the intrinsic rate of population growth for nilgai population was calculated as, r = 0.121, with 0 probability of extinction. Under 18% of females breeding scenario in the population the intrinsic growth rate was calculated as, r = -0.179, with probability of extinction of one and $t_{1/2}$ = ˜4 years. Under 9% of females breeding scenario in the population the intrinsic growth rate was calculated as, r = -0.287. with probability of extinction one and $t_{1/2}$= ˜ 3 years.

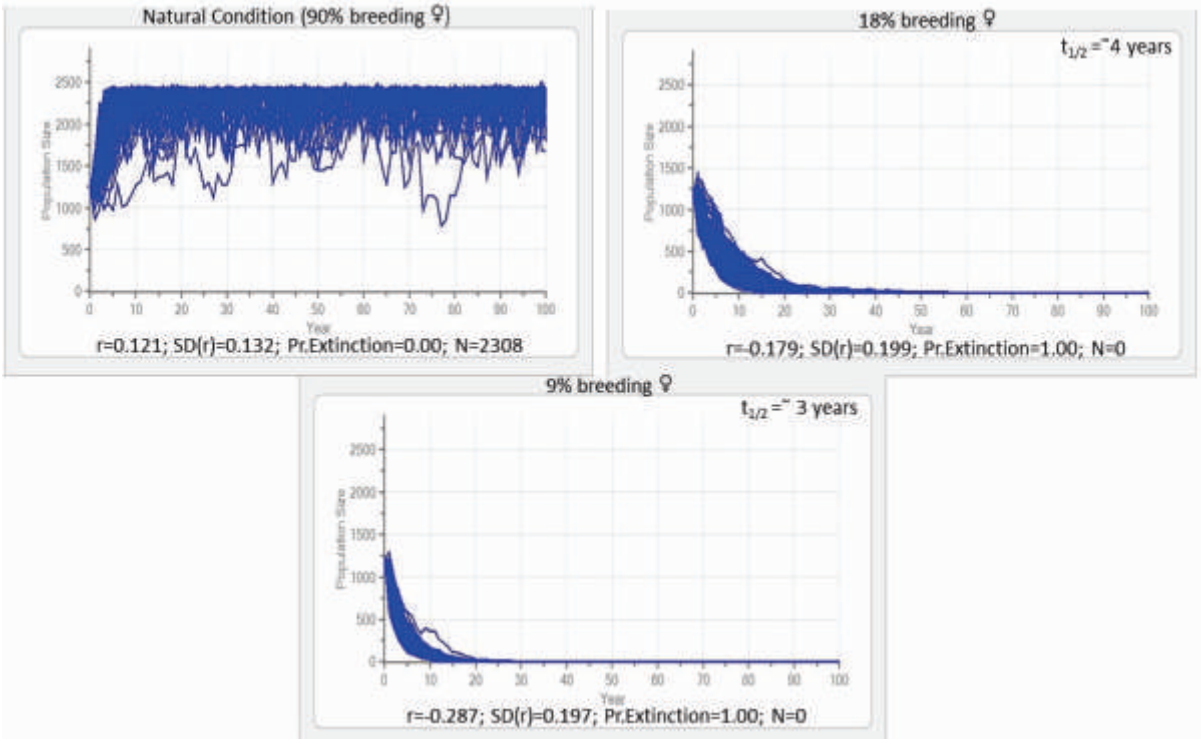


Figure 12.5 : *Population level scenarios for management interventions in Nilgai.*

Population control

The possibility of immuno-contraceptive use at large scale is not cost effective and logistically feasible in the field. It is suggested to do mass capture operation using boma technique. In some states it was earlier declared vermin and hunted for example Bihar, but operations were not successful at large scale. The captured nilgais should be used for augmentation in protected areas with low ungulate densities.

Population monitoring

Population where operation is carried out need to be monitored using camera trap based distance sampling for demographic response to interventions.

Appendix I

PRIMATE FACE IDENTIFICATION IN THE WILD

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Saket Anand[1], Qamar Qureshi[2] and
Yadvendradev Jhala[2]

Abstract : Ecological imbalance owing to rapid urbanization and deforestation has adversely affected the population of several wild animals. This loss of habitat has skewed the population of several non-human primate species like chimpanzees and macaques and has constrained them to co-exist in close proximity of human settlements, often leading to human-wildlife conflicts while competing for resources. For effective wildlife conservation and conflict management, regular monitoring of population and of conflicted regions is necessary. However, existing approaches like field visits for data collection and manual analysis by experts is resource intensive, tedious and time consuming, thus necessitating an automated, non-invasive, more efficient alternative like image based facial recognition. The challenge in individual identification arises due to unrelated factors like pose, lighting variations and occlusions due to the uncontrolled environments, that is further exacerbated by limited training data. Inspired by human perception, we propose to learn representations that are robust to such nuisance factors and capture the notion of similarity over the individual identity

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**Equal Contribution*

sub-manifolds. The proposed approach, Primate Face Identification (PFID), achieves this by training the network to distinguish between positive and negative pairs of images. The PFID loss augments the standard cross entropy loss with a pairwise loss to learn more discriminative and generalizable features, thus making it appropriate for other related identification tasks like open-set, closed set and verification. We report state-of-the-art accuracy on facial recognition of two primate species, rhesus macaques and chimpanzees under the four protocols of classification, verification, closed-set identification and open-set recognition.

Keywords: Face Recognition • Deep Learning • Primates • Social Good

Introduction

One of the key indicators of a healthy ecosystem is its constituent biodiversity. Over the last several decades, technological progress has substantially improved human quality of life, albeit at a cost of rapid environmental degradation. Specifically, to meet the needs of the growing human population, various factors like urban and infrastructural development, agricultural land expansion and livestock ranching have resulted in soaring rates of deforestation. In addition to the risk of extinction for many species, shrinking natural habitats have led to increased interactions between humans and wildlife, often raising safety concerns for both.

Conflicts with primarily forest-dwelling species like big cats (tigers, leopards, mountain lions, etc.), elephants, bears or wolves may cause severe injuries or even death to humans. On the other hand, there are species which have transitioned into a commensal relationship with humans, i.e., they rely on humans for food without causing direct harm. Due to their apparent harmlessness, several commensal (or semi-commensal) species like wild herbivores, wild boars, macaques and other non-human primates often dwell in close proximity of human settlements. This co-existence leads to indirect conflicts in the form of crop-raiding and property damage as well as occasional direct conflicts such as attacks or biting incidents. An example image of crop raiding and primates in close vicinity of humans is shown in Figure 2. Certain species like the rhesus macaque (*Macaca mulatta*) have become a cause of serious concern due to their resilience and ability to co-exist with humans in rural, semi-urban and urban areas. Their prolific breeding and short gestation periods lead to high population densities, thereby increasing the chances and extent of conflicts with humans. As a

Figure 1 : *Example images showing primates in human shared space and crop raiding [source: google images].*

consequence,organizations have resorted to lethal conflict management measures like culling [2], which become infeasible when the conflicted species have declining populations, e.g., the human-primate conflict crisis in Sri Lanka where two of the responsible primate species are endangered: Toque macaques (*Macaca sinica*) and the purple faced langur (*Trachypithecus vetulus*) [5]. Besides, the effectiveness of lethal measures is well debated and poorly designed initiatives could have unexpected consequences like increased aggression or even extinction of the conflicted species [16]. On the other hand, non-lethal approaches are easier to adopt across geographies as they avoid complex socio-religious issues [19]. Two recurring non-lethal themes in conflict management discussions are population monitoring and stakeholder engagement [16], both of which can be easily achieved with a combination of smartphone and AI technology. Pursuing a crowdsourcing approach to population monitoring and conflict reporting has two direct benefits: the cost and scalability of data collection for population monitoring can be improved drastically and active involvement of the affected community can help increase awareness, which in turn abates the human behavioral factors that often influence human-wildlife conflicts.

In this work, we focus on addressing the human-primate conflicts, largely because of the frequency and magnitude of encounters in urban, rural and agricultural regions across developing South Asian nations [1]. Inspired by the success and scalability of human face recognition, we propose a Primate Face Identification (PFID) system. Automatic identification capabilities could serve as a backbone for a crowdsourcing platform, where geo-referenced images submitted by users are automatically indexed by individuals, gender, age, etc. Such an indexed database could simplify downstream tasks like primate population monitoring and analysis of conflict reports, enabling better informed and effective strategies for conflict as well as conservation management. We summarize the contributions of this paper as follows:

– We propose *Primate Face Identification* (PFID), a deep neural network based system for automated identification of individual primates using facial images.
– We introduce a guided pairwise loss using similar and dissimilar image pairs to learn robust and generalizable representations.
– Our fully automatic pipeline convincingly beats state-of-the-art methods on two datasets (macaques and chimpanzees) under *all* settings.

Existing Work on Face Recognition

There is a vast body of literature in human face recognition. Without attempting to present a comprehensive survey, we briefly discuss prior work relevant to facial identification of primates. We broadly categorize these approaches into two categories: Non Deep Learning Approaches and Deep Learning Approaches.

Non Deep Learning Approaches Traditional face recognition pipelines comprised of face alignment, followed by low level feature extraction and classification. Early works in primate face recognition [13], adapted the Randomfaces [25] technique for identifying chimpanzees in

the wild and follows the standard pipeline for face recognition. Later, LemurID was proposed in [6], which additionally used manual marking of the eyes for face alignment. Patch-wise multi-scale Local Binary Pattern (LBP) features were extracted from aligned faces and used with LDA to construct a representation, which was then used with an appropriate similarity metric for identifying individuals.

Deep Learning Approaches Freytag et al. [9] used Convolutional Neural Networks (CNNs) for learning a feature representation of chimpanzee faces. For increased discriminative power, the architecture uses a bilinear pooling layer after the fully connected layers (or a convolutional layer), followed by a matrix log operation. These features are then used to train an SVM classifier for classification of known identities. Later, [4] developed face recognition for gorilla images captured in the wild. This approach finetuned a YOLO detector [17] for gorilla faces. For classification, a similar approach was taken as [9], where pre-trained CNN features are used to train a linear SVM. More recently, [7] proposed PrimNet, a deep neural net based approach that uses the *Additive Margin Softmax* loss [22] and achieves state of the art performance for identifying individuals across different primate species including lemur, chimpanzee and golden monkey. However, it requires substantial manual effort to designing landmark templates for face alignment prior to identification process, which can adversely affect adoption rates in a crowdsourced mobile app setting. For human face recognition techniques, various approaches have improved performance by combining the standard cross entropy loss with other loss functions such as contrastive loss [21] and center loss [23] to learn more discriminative features.

Primate Face Identification (PFID) System

Pose Invariant Representation Learning We would like to motivate the choice of our loss function with the following reasons

1. Our approach is inspired by the human perception system, which is robust to nuisance factors like illumination and pose and is able to identify individual faces captured in unconstrained environments and extreme poses. Geometrically, face images of an identity defines a sub-manifold [15] in image manifold of faces. This allows one to devise a metric such that sample pairs of the same identity have small distances regardless of pose and other nuisance factors, while those of different identities have larger distances. In PFID, we use a deep neural network to learn such a representation through a specially designed loss function over similar and dissimilar pairs of primate face images.

2. Learning invariant features has long been a challenging issue in computer vision. Owing to the high curvature of original image data manifold [14] , simple metric like euclidean distance fails to capture the underlying data semantics. Consequently, linear methods also are inappropriate to learn decision boundaries for tasks like image recognition. In such scenarios, deep learning approaches have come in handy, with their ability to flatten the data manifold owing to the successive non linear operations applied though

a series of layers [3]. However, deep models are often trained with a cross-entropy based classification loss, to drive the class probability distribution for a given image independently to one hot encoding vector. Given sufficient training data, this training protocol often generalizes well for classification task, however, its performance is often limited on other related tasks like verification and unseen class generalizabilty. The latter's performance crucially depends on the ability to learn a representation space that can model class-level similarities. By incorporating a pairwise similarity loss term operating on the class probability (softmax) distributions, we drive the learned representations to be semantically more meaningful, and hence invariant to other factors.

We now present our proposed PFID loss function for unique identification of primates using cropped facial images that can be obtained using state of the art deep learning based detectors. We note that images will be largely collected by the general public, professional monkey catchers and field biologists. Typically, we expect the images to be captured in uncontrolled outdoor scenarios, leading to significant variations in facial pose and lighting. These conditions are challenging for robust eye and nose detection, which need to be accurate in order to be useful for facial alignment. Consequently, we train our identification model to work without facial alignment and capture the semantic similarities of the underlying space.

The proposed loss formulation combines the standard cross entropy network with a guided pairwise KL divergence loss imposed on similar and dissimilar pairs. Using pairwise loss terms ensure that the underlying features are more discriminative and generalize better. Our analysis in Sec. 4.4 show empirical evidence that the learned features are more clusterable than when

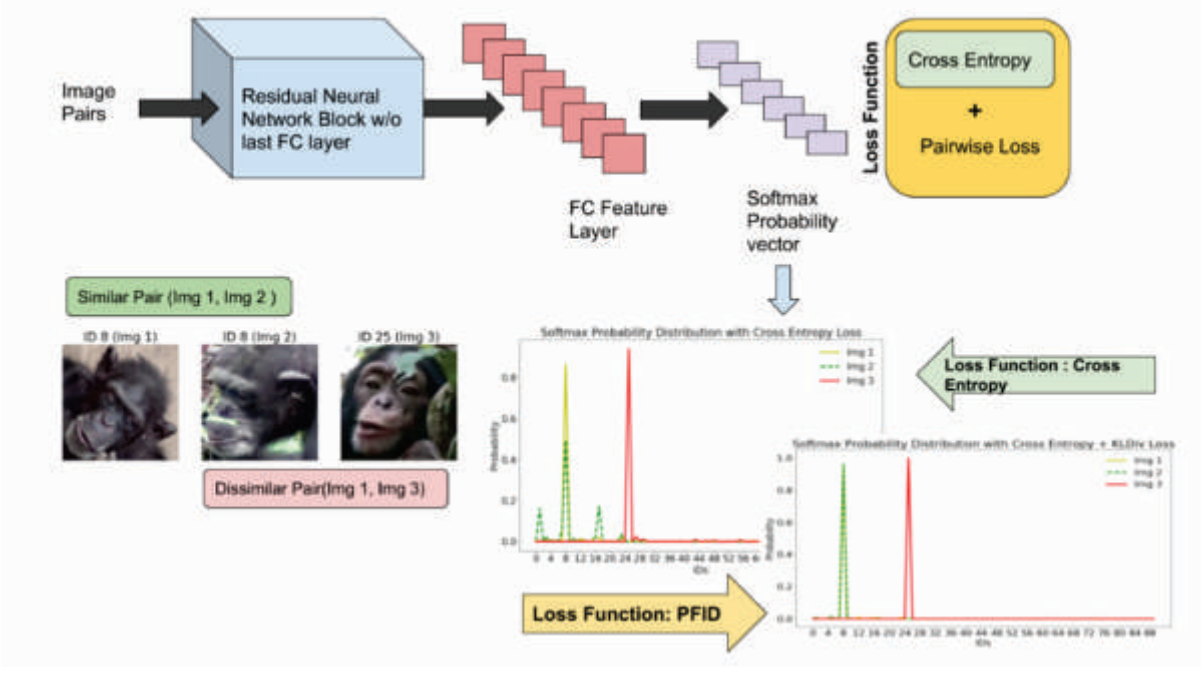

Figure 2: *Illustration of proposed PFID loss function vs. the standard cross entropy loss on the learned class probability distributions with ResNet model.*

trained with the standard cross-entropy loss.

An illustration of the effect of loss function is shown in Figure 2. A similar pair corresponds to images of same individual, while a dissimilar pair corresponds to images from two different identities. The learned class probability distribution for a similar pair and dissimilar pair using two different loss functions is shown. In case of network trained with PFID loss, the class probabilities are maximally similar for a similar pair as oppose to standard cross entropy loss.

Let, X = $\{x_1, x_2, ..., x_n\}$ be the training dataset of n samples with $l_i \in \{1, 2, ..., K\}$ as the associated labels. We use the labeled training data to create sets of similar image pairs, $C_s = \{(i,j): x_i, x_j \in X, l_i = l_j\}$, and that of dissimilar pair, $C_d = \{(i,j): x_i, x_j \in X, l_i 6 = l_j\}$ for $i, j \in \{1, 2, \cdots, n\}$. The KL divergence between two distribution p^i and q^j corresponding to points x_i and x_j is given by

$$KL(p^i||q^j) = \sum_{k=1}^{K} p_k^i \log \frac{p_k^i}{q_k^j} \tag{1}$$

For a similar pair $(i,j) \in C_s$, we use the symmetric variant of (1) given by

$$\mathcal{L}_s^{ij} = KL(p^i||q^j) + KL(q^j||p^i) \tag{2}$$

and for a dissimilar pair $(i,j) \in C_d$, we use its large-margin variant for improving discriminative power

$$\mathcal{L}_d^{ij} = \max(0, m - KL(p^i||q^j)) + \max(0, m - KL(q^j||p^i)) \tag{3}$$

Where m is the desired margin width between dissimilar pairs. It is important to note that during training, when both x_i and x_j are misclassified by the model, minimizing (2) may lead to an increase in the bias.

Guided Pairwise Loss Function Since we use class labels for the cross-entropy loss, we incorporate them in the pairwise loss terms to guide the training. Subsequently, we modify the terms in (2) and (3) to get the following guided KL divergence loss term

$$L_s = X\ aL_{ijs},\ L_d = X\ aL_{ijd}\ _{i,j \in C_s}\ _{i,j \in C_d} \tag{4}$$

Where, $a = 1$ if either $\text{argmax}\ p^i = l_i$ or $\text{argmax}\ q^j = l_j$ and $a = 0$ otherwise. The loss function for PFID is given by the sum of standard cross entropy (L_{CE}) and the guided KL divergence loss

$$\mathcal{L}(\theta) = \mathcal{L}_{CE} + \frac{1}{|C_s|} \sum_{j,k \in C_s} a\mathcal{L}_s^{jk} + \frac{1}{|C_d|} \sum_{j,k \in C_d} a\mathcal{L}_d^{jk} \tag{5}$$

This loss function is used to train the network with a mini-batch gradient descent. Here $|C_s|$ and $|C_d|$ are the number of similar and dissimilar pairs respectively in a given batch. More details on the training are provided in Sec. 4.3.

Experimental Setup and Results

Dataset

We evaluate our model using three datasets, the details of which are given in Table 1. As is typical of wildlife data collected in uncontrolled environments, all the three datasets have a significant class imbalance as reported in the Table 1.

Rhesus Macaque Dataset The dataset is collected using DSLRs in their natural dwelling in an urban region in the state of Uttarakhand in northern India. The dataset is cleaned manually to remove images with no or very little facial content (e.g., extreme poses with only one ear or only back of head visible). The filtered dataset has 59 identities with a total of 1399 images. An illustrative set of pose variations for the datasets are shown using the cropped images in figure 3. Due to the small size of this dataset, we combined our dataset with the publicly available dataset by Witham [24]. The combined dataset comprises 7679 images of 93 individuals. Note that we use the combined dataset only for the individual identification experiments, as the public data by Witham comprises of pre-cropped images. On the other hand, the detection and the complete PFID pipeline is evaluated on a test set comprising full images from our macaque dataset.

Chimpanzee Dataset The C-Zoo and C-Tai dataset consists of 24 and 66 individuals with 2109 and 5057 images respectively [9]. The C-Zoo dataset contains good quality images of chimpanzees taken in a Zoo, while the C-Tai dataset contains more challenging images taken under uncontrolled settings of a national park. We combine these two datasets to get 90 identities with a total of 7166 images.

Table 1: Dataset Summary. The numbers in the brackets show the range of samples per individual ([min,max]), highlighting the imbalance in the datasets.

Dataset	Rhesus Macaques	C-Zoo	C-Tai
# Samples	7679	2109	5057
# Classes	93	24	66
# Samples/individual	[4,192]	[62,111]	[4,416]

Figure 3: *Pose variations for one of the Rhesus Macaque (Top) and Chimpanzee (Below) from the dataset.*

Evaluation Protocol

We evaluate and compare the performance of our PFID system under four different experimental settings, namely: classification, closed-set identification, open-set identification and verification.

Classification To evaluate the classification performance the dataset is divided into 80/20 train/test splits. We present the mean and standard deviation of classification accuracy over five stratified splits of the data. As opposed to other evaluation protocols discussed below, all the identities are seen during the training, with unseen samples of same identities in the test set.

Open and Closed-Set Identification Both, closed-set and open-set performance is reported on *unseen* identities. We perform 80/20 split of data w.r.t. to identities, which leads to a test set with 18 identities in test for both chimpanzee and macaque datasets. We again use five stratified splits of the data. For each split, we further perform 100 random trials for generating the probe and gallery sets. However, the composition of the probe and gallery sets for the closed-set scenario is different from that of open set. Closed-Set: In case of closed-set identification, all identities of images present in the probe set are also present in the gallery set. Each probe image is assigned the identity that yields the maximum similarity score over the entire gallery set. We report the fraction of correctly identified individuals at Rank-1 to evaluate the performance.

Open-Set: In case of open-set identification, some of the identities in the probe set may not be present in the gallery set. This allows to evaluate the recognition system to validate the presence or absence of an identity in the gallery. To validate the performance, from the test of 18 identities, we used all the images of odd numbered identities as probe images with no images in the gallery. The rest of the even numbered identities are partitioned in the same way as closed-set identification to create probe and gallery sets. We report Detection and Identification Rate (DIR) at 1% FAR to evaluate open-set performance.

Verification We compute positive and negative scores for each sample in test set. The positive score is the maximum similarity score of the same class and negative scores are the maximum scores from each of the classes except the true class of the sample. In our case, where the test data has 18 identities, each sample is associated with a set of 18 scores, with one positive score from the same identity and 17 negative scores corresponding to remaining 17 identities. The verification accuracy is reported as mean and standard deviation at 1% False Acceptance Rates (FARs).

Network Details and Parameter Setting

We resize all the face images in macaque and chimpanzee dataset to 112 × 112. We add the following data augmentations: random horizontal flips and random rotations within 5 degrees for both the datasets. We use the following base network architectures for PFID: ResNet-18

[10] and DenseNet-121 [11] and remove the first maxpool layer because of small image size. For CE setting, we fine-tuned the imagenet pre-trained networks with cross-entropy loss and a batch size of 16. For the PFID setting, for each image in a batch, a similar class image is sampled to make a batch size of 8 pairs (16 images in a batch). The dissimilar pairs are then exhaustively created from these pairs. We used SGD for optimization with an initial learning rate of 10^{-3} and weight decay of $5e-4$. We trained all the models for both datasets for 40 epochs with learning rate decay by 0.1 at 25^{th} and 35^{th} epoch. We observed better performance with batch size of 16 instead of 32 or higher especially in case of training with only cross-entropy loss. It is recommended to use a lower batch size given that the training data is less in both the datasets.

Results

We present the results corresponding to PFID and other state of the art approaches for face recognition.

Baseline Results For the baseline results, we extracted the penultimate (FC) layer features from both ResNet-18 and DenseNet-121 models. For all the evaluation protocols, the features are *l*2-normalized and in addition for classification, they are used to train a SVM (Support Vector Machine) classifier by performing a grid-search over the regularization parameter. The results are given in the first 2 rows of the Table 2 and 3. We directly used the features and did not perform PCA (Principal Component Analysis) to reduce the number of feature dimensions because it had no impact on the performance in each evaluation.

Comparison with state of the art approaches We compare PFID with recent work PrimNet [7] that achieved state of the art performance on chimpanzee face dataset. While our approach outperforms PrimNet by a large margin, it is worth noting that our results are reported on non-aligned face images, that makes PFID better suited for the application of crowdsourced population monitoring by eliminating the need for manual annotations of fiducial landmarks.

Table 2: Evaluation of Chimpanzee dataset for classification, closed-set, open-set and verification setting. Baseline results are reported by taking the penultimate layer features of the network and training a SVM for classification. For all the remaining settings the features are directly used for the evaluation protocol.

Method	Classification Rank-1	Closed-set Rank-1	Open-set Rank-1	Verification 1 % FAR
Baseline (ResNet-18 FC + SVM)	55.38 ± 1.18	70.51 ± 2.98	12.80 ± 5.73	37.10 ± 4.63
Baseline (DenseNet-121 FC +SVM)	61.78 ± 1.4	75.34 ± 3.98	30.51 ± 6.61	54.80 ± 3.65
ArcFace (ResNet-50)	85.47 ± 0.86	78.47 ± 5.81	41.24 ± 7.82	63.91 ± 5.37
SphereFace-20	78.38 ± 1.23	72.72 ± 3.44	35.49 ± 8.34	57.74 ± 6.38
PrimNet	70.86 ± 1.19	72.22 ± 5.33	37.27 ± 5.48	62.83 ± 5.98
CE (ResNet-18)	85.29 ±1.43	86.44 ± 5.42	48.62 ± 9.05	75.19 ± 8.16
CE (DenseNet-121)	86.74 ± 0.74	87.01± 5.39	53.60 ± 13.04	76.86 ± 9.55
PFID (ResNet-18)	88.98 ± 0.26	88.26 ± 5.01	59.36 ± 9.12	80.06 ± 6.62
PFID (DenseNet-121)	90.78 ± 0.53	91.87 ± 2.92	66.24 ± 8.08	83.23 ± 6.07

Since ResNet-18 and DenseNet-121 are pretrained on imagenet data, we additionally fined-tuned ArcFace [8] and SphereFace [12] models that are pre-trained on human face images, specifically on CASIA [26] dataset. We use ResNet-50 as the backbone for ArcFace and 20-layer network for SphereFace, and use the parameters given in the respective papers. We observed best performance with batch size 32 in all the three methods. We used a learning rate of 0.1, 0.01 and 0.001 for PrimNet (trained from scratch), SphereFace and ArcFace respectively and weight decay as $5e-4$. We trained all the models for 30 epochs to avoid over-fitting with learning rate decay by 0.1 at 15^{th} and 25^{th} epoch. The results are reported in Table 2 and 3 for both the datasets. The results highlight that the imagenet pre-trained models generalize well in our case where the training data is not huge. Further, it should be noted that the results reported for the three models ArcFace, SphereFace and PrimNet are also reported without face alignment as oppose to the results reported in the respective papers. While we report results with non-aligned face images, we would also like to point out that the performance dropped in all the approaches with aligned face images in case of chimpanzee dataset owing to loss of features in aligned faces.

PFID Results To show the efficiency of our approach, we fine-tuned ResNet-18 and DenseNet-121 models with standard cross entropy (CE) loss and report in the Table 3 and 2 for macaque and chimpanzee datasets respectively and compared it with the PFID loss. We observe an increase in performance for the four evaluation protocols with PFID loss as opposed to traditional cross entropy fine-tuned network. Imposing a KL-divergence loss has improved the discriminativeness of features by skewing the probability distributions of similar and dissimilar pairs. For chimpanzee dataset an improvement of 4.04%, 4.86 %, 12.64% and 6.97 % is achieved in case of classification, closed-set, open-set and verification respectively using DenseNet-121. The corresponding CMC (Cumulative Matching Characteristic) and TAR (True Acceptance Rate) vs FAR plots for the datasets are shown in Figure 4.

Table 3: Evaluation of Rhesus Macaque dataset for classification, closed-set, open-set and verification setting. Baseline results are reported by taking the penultimate layer features of the network and training a SVM for classification. For all the remaining settings the features are directly used for the evaluation protocol.

Method	Classification Rank-1	Closed-set Rank-1	Open-set Rank-1	Verification 1 % FAR
Baseline (ResNet-18 FC +SVM)	85.28 ± 0.25	88.29 ± 2.95	50.09 ± 7.35	66.98 ± 9.21
Baseline (DenseNet-121 FC +SVM)	88.3 ± 0.57	89.24 ± 3.63	53.93 ± 10.27	71.34 ± 8.88
ArcFace (ResNet-50)	98.23 ± 0.47	93.98 ± 2.99	67.07 ± 13.91	95.16 ± 1.56
SphereFace-20	97.61 ± 0.74	93.41 ± 2.19	95.62 ± 12.21	93.18 ± 1.95
PrimNet	97.11 ± 0.65	90.94 ± 2.54	65.98 ± 15.23	92.14 ± 2.82
CE (ResNet-18)	97.91 ± 0.58	95.94 ± 2.94	79.69 ± 8.12	96.35 ± 2.06
CE (DenseNet-121)	97.99 ± 0.69	96.24 ± 0.85	71.36 ± 10.05	96.01 ± 3.01
PFID (ResNet-18)	98.71 ± 0.41	96.18 ± 1.58	83.02 ± 7.36	97.71 ± 0.91
PFID (DenseNet-121)	98.91 ± 0.40	97.36 ± 1.73	84.00 ± 7.43	98.24 ± 0.94

Feature Learning and Generalization To further show the effectiveness of PFID loss function and robustness of features, we perform cross dataset experiments in Table 5. We used model trained on chimpanzee dataset and extracted features on macaque dataset to evaluate the performance for closed-set, open-set and verification task and vice versa. We compared the quality of the features learned with PFID with the features learned with cross entropy based fine-tuning. We also show the generalizability between two chimpanzee datasets captured in different environments i.e. CZoo and CTai. The results clearly highlight the advantage of PFID over cross entropy loss for across data generalization. Additionally, to highlight the discriminativeness and clusterability of the class specific features, we cluster the feature representations of unseen (identities) test data using K-means clustering algorithm. We report the clustering performance in Table 4 and compare with the standard cross entropy loss.

Table 4: Comparison of K-means clustering performance on the learned representations with DenseNet-121. The results highlight that the PFID learns more clusterable space.

Model	Macaque	Chimpanzee
	NMI	NMI
CE	0.868 ± 0.008	0.686 ± 0.084
PFID	0.897 ± 0.030	0.715 ± 0.089

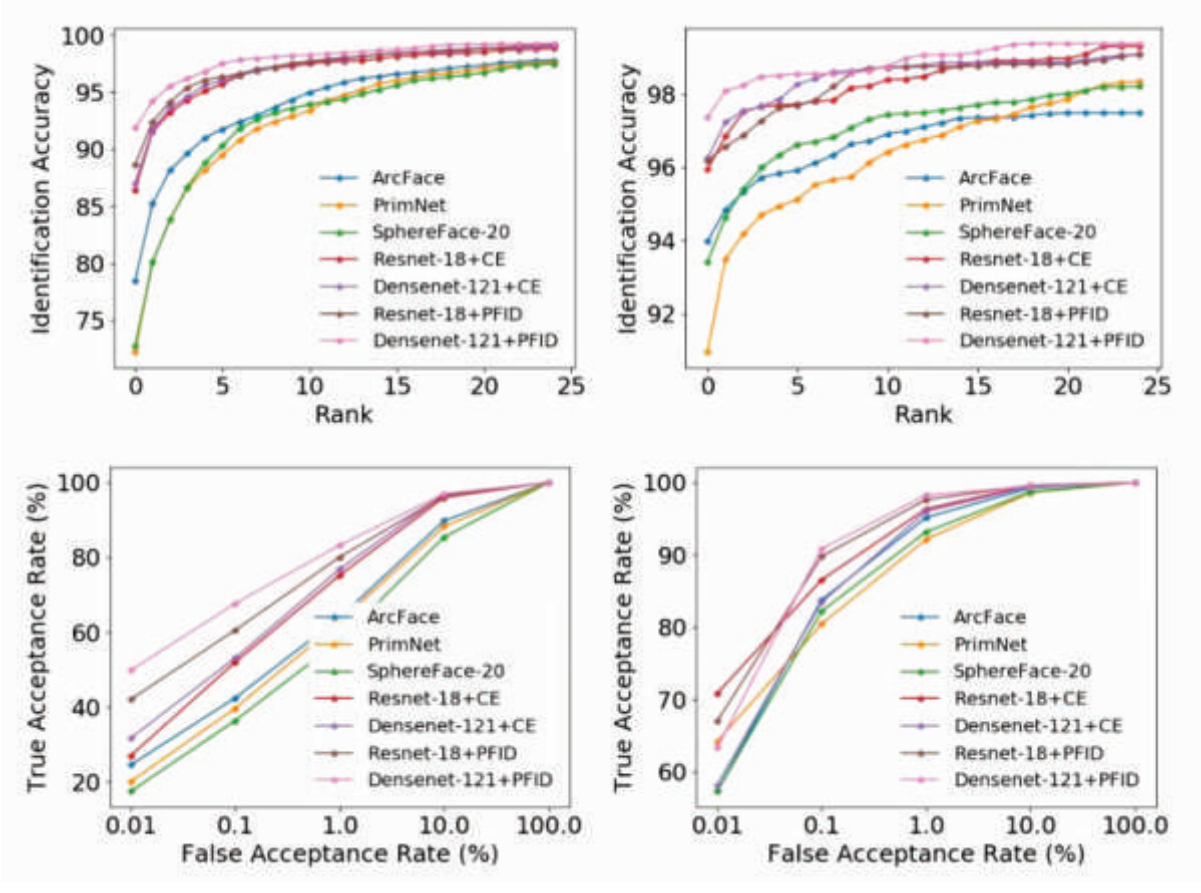

Figure 4 : *CMC (Top) and TAR vs FAR (Bottom) plots for (Left) C-Zoo+CTai and (Right) Rhesus Macaques dataset.*

Table 5: Evaluation of learned model across datasets. Left of the arrow indicates the dataset on which the model was trained on, and right of the arrow indicates the evaluation dataset. All the results are reported for DenseNet-121 network.

	Macq. → Chimp.		Chimp. → Macq.		CZoo → CTai		CTai → CZoo	
	CE	PFID	CE	PFID	CE	PFID	CE	PFID
Closed Set	54.58	63.48	83.02	88.38	59.92	70.35	87.54	91.96
Open Set	13.56	34.29	32.04	43.00	17.21	27.21	43.25	64.75
Verification	43.02	63.77	67.51	75.37	48.68	60.57	66.71	82.22

Comparison with Siamese Network based features One might draw similarity of our approach with the popular siamese networks [20] that are trained on similar and dissimilar pairs to result in a similairty score at the output. We train ResNet-18 on chimpanzee data in siamese setting with pairwise hinge-loss on features to show that the learned features in the classification setting are not discriminative as compared to our PFID. While training in siamese setting, we also observe that the network overfits on the training data and performs poorly on unseen classes. The results for different evaluation protocols are: Classification (83.97 ± 1.42), Closed-set (75.45 ± 5.51), Verification (57.28 ± 7.37) and Open-set (22.22 ± 8.07).

Identification on Detected Face Images The above results evaluated the performance of PFID on cropped face images *i.e.* the true bounding box of the test samples. As the captured images with handheld devices like cameras would also have background, we evaluate the performance of PFID on the detected faces on test samples. Since, we had 1191 full images for the Macaque dataset, the detector is trained and tested with a split of 80/20. We fine-tune state-of-the-art Faster-RCNN [18] detector for detecting macaque faces and achieve highly accurate face detection performance. The identification results on the cropped faces obtained from the detector is shown in Table 6. For identification evaluation, we have 10 identities and 227 images for both closed-set and verification, whereas for open-set we extend the probe set by adding 8 identities and 1100 samples which are not part of the dataset.

Integration with Crowd Sourcing App

We have developed a simple app to work as a front-end for PFID, which permits a user to upload geo-tagged images of individuals and troops as well as report a conflict incident. Augmented with the PFID based back-end service, this app could help maintain an updated database of reported conflicts, along with a primate database indexed by individuals, troop and last-sighted locations, which can be used with techniques like Capture-Recapture to estimate population densities.

Table 6: Evaluation of detected macaque faces for closed set, open set and verification setting.

Method	Closed-set Rank-1	Open-set Rank-1	Verification 1 % FAR
CE (ResNet-18)	95.00	70.78	89.22
PFID (ResNet-18)	97.20	78.80	91.11
CE (DenseNet-121)	95.30	80.67	91.56
PFID (DenseNet-121)	97.80	89.67	95.11

Conclusion

In this work, we discussed the problem of unique identification of non-human primates using face images captured in the wild. From existing literature, we found that population monitoring is an important step in the management strategies and largely rely only on field-based efforts. In this work, we identified this challenge and proposed an alternate solution that can simultaneously improve monitoring of commensal primates as well as actively involve the affected human community without any serious cost implications. We developed a novel face identification approach that is capable of learning pose invariant features, thus allowing to generalize well across poses without the requirement of a face alignment step. Additionally, the proposed approach leverages the pairwise constraints to capture underlying data semantics enabling it to perform effectively for unseen classes. With the effectiveness of our approach in different identification tasks on real world data, we foresee that the PFID system could become a part of widely used wildlife management tools like SMART[1].

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Appendix II

Table 2.1 : Sinuosity Index of radio-collared resident elephants

Individual	Winter							Summer						
	cv	mean speed	sd_speed	sinuosity	Emax	min delta S	min c	cv	mean speed	sd_speed	sinuosity	Emax	min delta S	min c
Meera 2	1.152	8895.731	10253.7	0.133	0.3752	10	-0.075	1.901	12298.640	23363.810	0.121	0.224	13	-0.035
Akansha 2	1.231	6889.399	8245.336	0.142	0.5494	10	-0.081	2.136	11312.090	24179.340	0.118	0.387	11	-0.062
Ananya 2	1.822	6489.622	11836.7	0.150	0.4724	11	-0.050	1.387	10670.680	14802.120	0.130	0.248	9	-0.047
Usha	1.459	12811.84	18756.01	0.105	0.4907	6	-0.224	1.170	11608.000	13512.340	0.123	0.286	11	-0.048
Makhna	1.087	7016.766	7646.49	0.171	0.1444	7	-0.085	1.209	6928.922	8375.603	0.172	0.111	12	-0.039
Aiyappa	1.629	6686.862	6410.793	0.177	0.1129	10	-0.136	1.771	10507.600	18625.510	0.134	0.188	9	-0.074
Beetamma	1.912	10413.09	20899.37	0.138	0.1376	10	-0.024	1.159	10593.990	14064.260	0.141	0.082	7	-0.038
Bhuveneshwari	1.225	10532.97	19990.56	0.137	0.1571	10	-0.059	1.555	10958.160	17193.130	0.139	0.085	9	-0.040
Oldbelt	1.290	8899.934	11537.61	0.142	0.2476	10	-0.057	1.498	8437.635	12625.470	0.155	0.126	15	-0.021
NE2	1.589	12592.42	19968.35	0.101	0.6546	15	-0.053	1.105	10144.390	11207.150	0.122	0.455	12	-0.026
NE4	1.152	8083.364	9305.853	0.131	0.5697	11	-0.023	1.068	9474.802	10179.440	0.136	0.269	16	-0.013
Individual	**South-West Monsoon**							**North-East Monsoon**						
	cv	mean speed	sd_speed	sinuosity	Emax	min delta S	min c	cv	mean speed	sd_speed	sinuosity	Emax	min delta S	min c
Meera 2	1.323	9426.153	12476.660	0.140	0.211	13	-0.027	1.424	8536.753	12146.520	0.137	0.375	11	-0.045
Akansha 2	1.280	11447.780	14643.690	0.124	0.247	17	-0.033	1.373	7822.562	10745.530	0.138	0.447	14	-0.071
Ananya 2	1.529	8048.375	12304.310	0.142	0.352	12	-0.038	1.447	7008.406	10144.570	0.142	0.514	14	-0.044
Usha	1.355	9663.037	13117.460	0.125	0.417	11	-0.068	0.844	4894.045	4121.216	0.161	0.697	30	-0.086
Makhna	1.164	7741.278	9595.019	0.166	0.075	9	-0.040	1.020	14324.110	14566.230	0.106	0.332	7	-0.046
Aiyappa	1.559	9263.983	14409.050	0.148	0.118	8	-0.038	NA	NA	NA	NA	NA	NA	NA
Beetamma	1.239	10544.900	13199.800	0.139	0.126	7	-0.038	1.271	11501.840	14652.110	0.131	0.148	10	-0.045
Bhuveneshwari	1.223	9431.023	11613.150	0.148	0.106	11	0.033	1.290	11487.490	14880.070	0.133	0.119	9	-0.060
Oldbelt	1.173	8640.678	10128.300	0.150	0.157	10	-0.023	1.285	9052.670	11800.160	0.150	0.121	11	-0.041
NE2	1.109	7765.341	8606.665	0.134	0.501	34	-0.028	1.498	9271.741	13885.260	0.122	0.526	26	-0.029
NE4	1.409	6035.940	8640.199	0.164	0.352	31	-0.032	1.235	5586.053	6959.852	0.161	0.491	41	-0.021

Emax= Dimensionless estimate of the maximum expected displacement of the trajectory, min_delta S= mean of sine of turning angles, min_c= mean of cosine of turning angles, cv= coefficient of variation of step length.

SINUOSITY GRAPHS OF RESIDENT RADIO-COLLARED ELEPHANTS

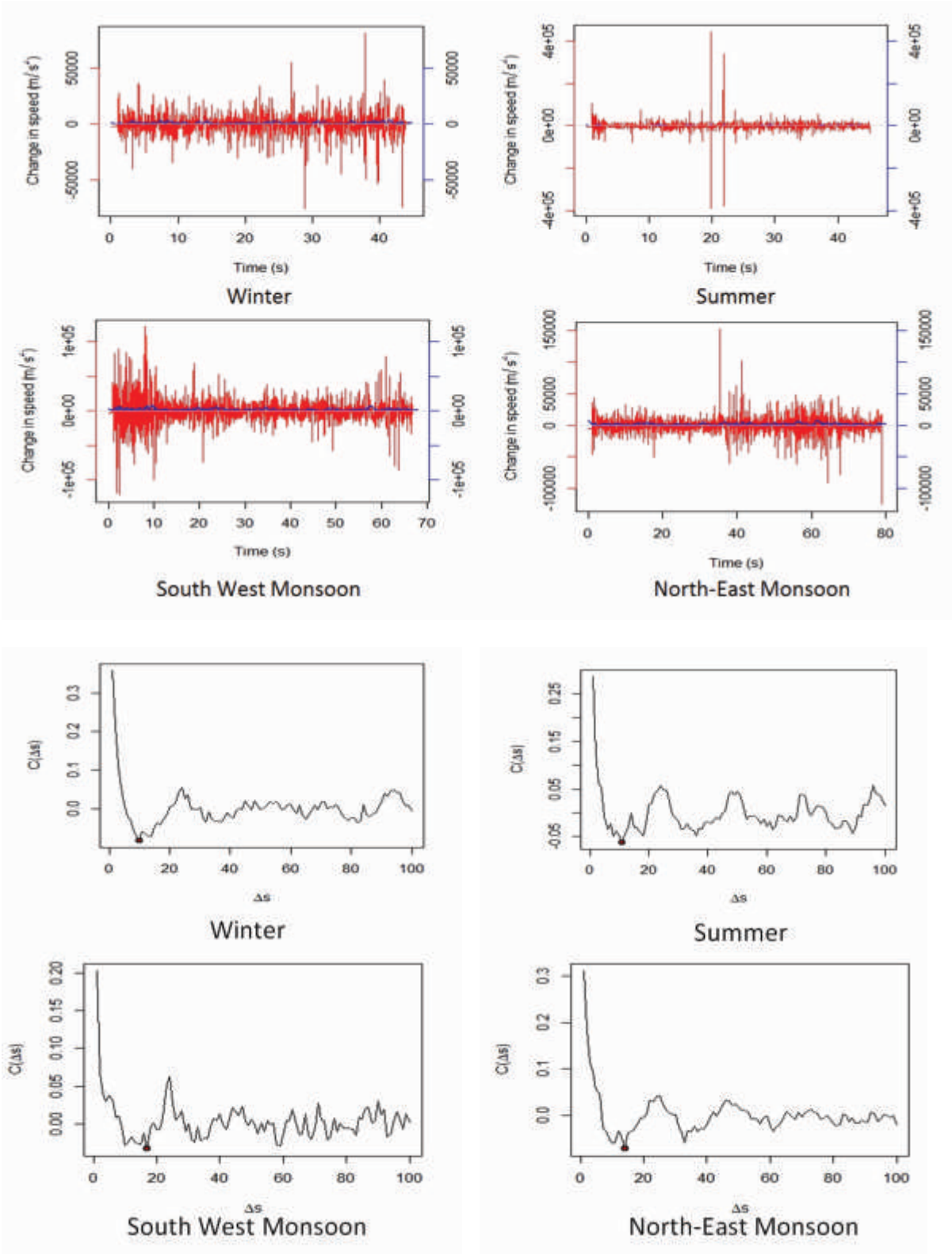

Figure 2.1: *Acceleration, speed and Direction autocorrelation of trajectory of Akansha with respect to season*

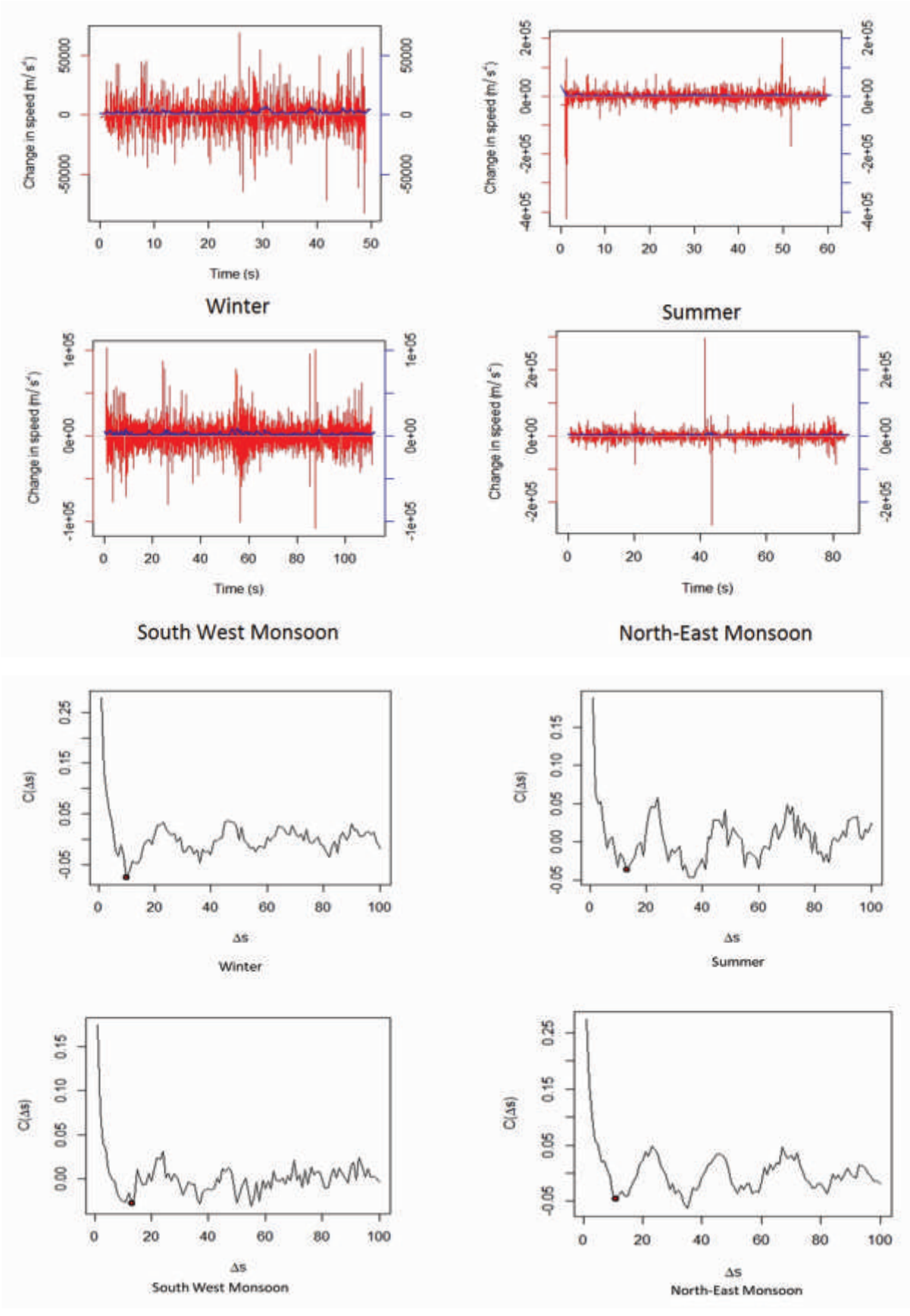

Figure 2.2 : *Acceleration, speed and Direction autocorrelation of trajectory of Meera with respect to season*

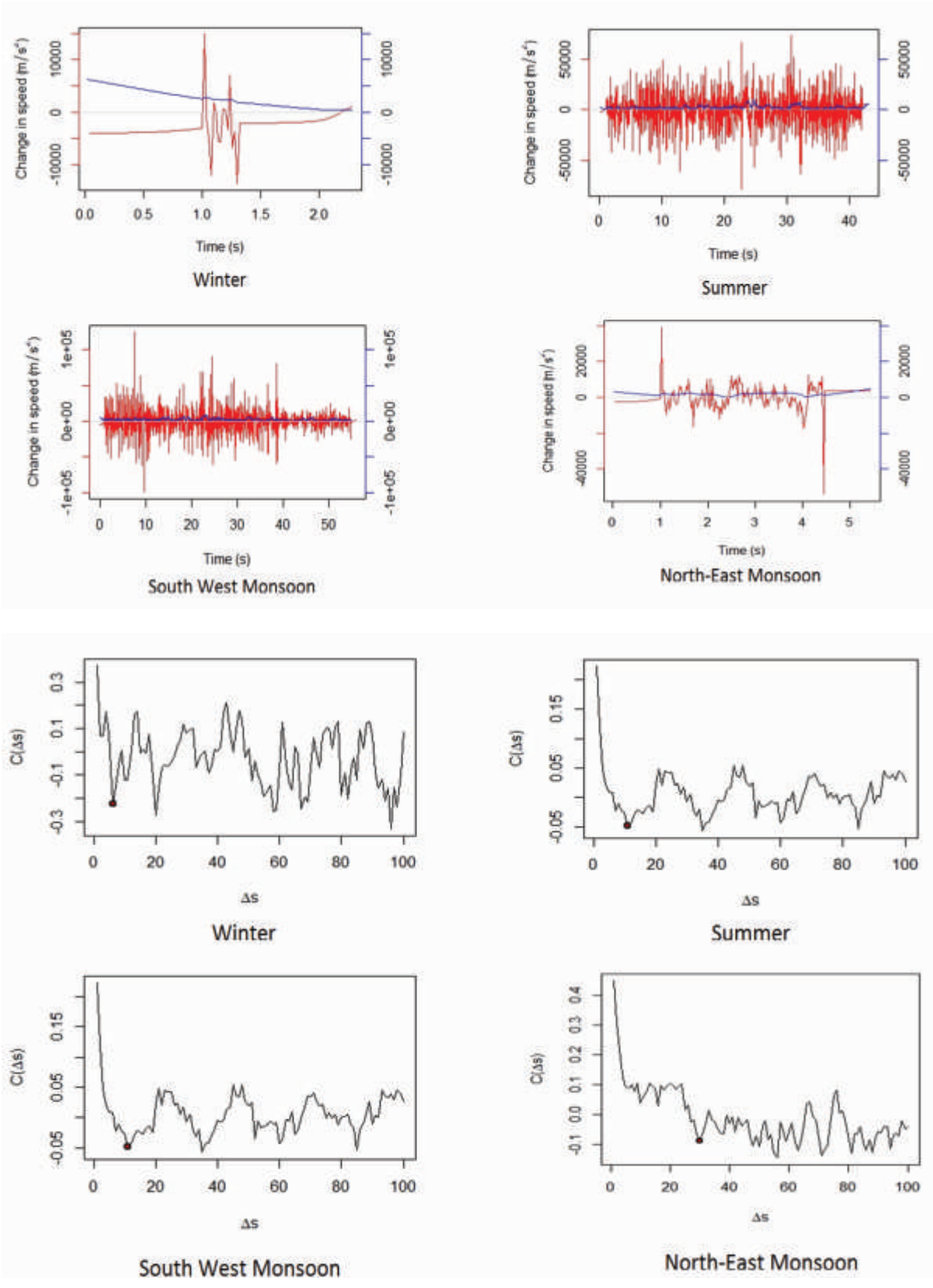

Figure 2.3 : *Acceleration, speed and Direction autocorrelation of trajectory of Usha with respect to season*

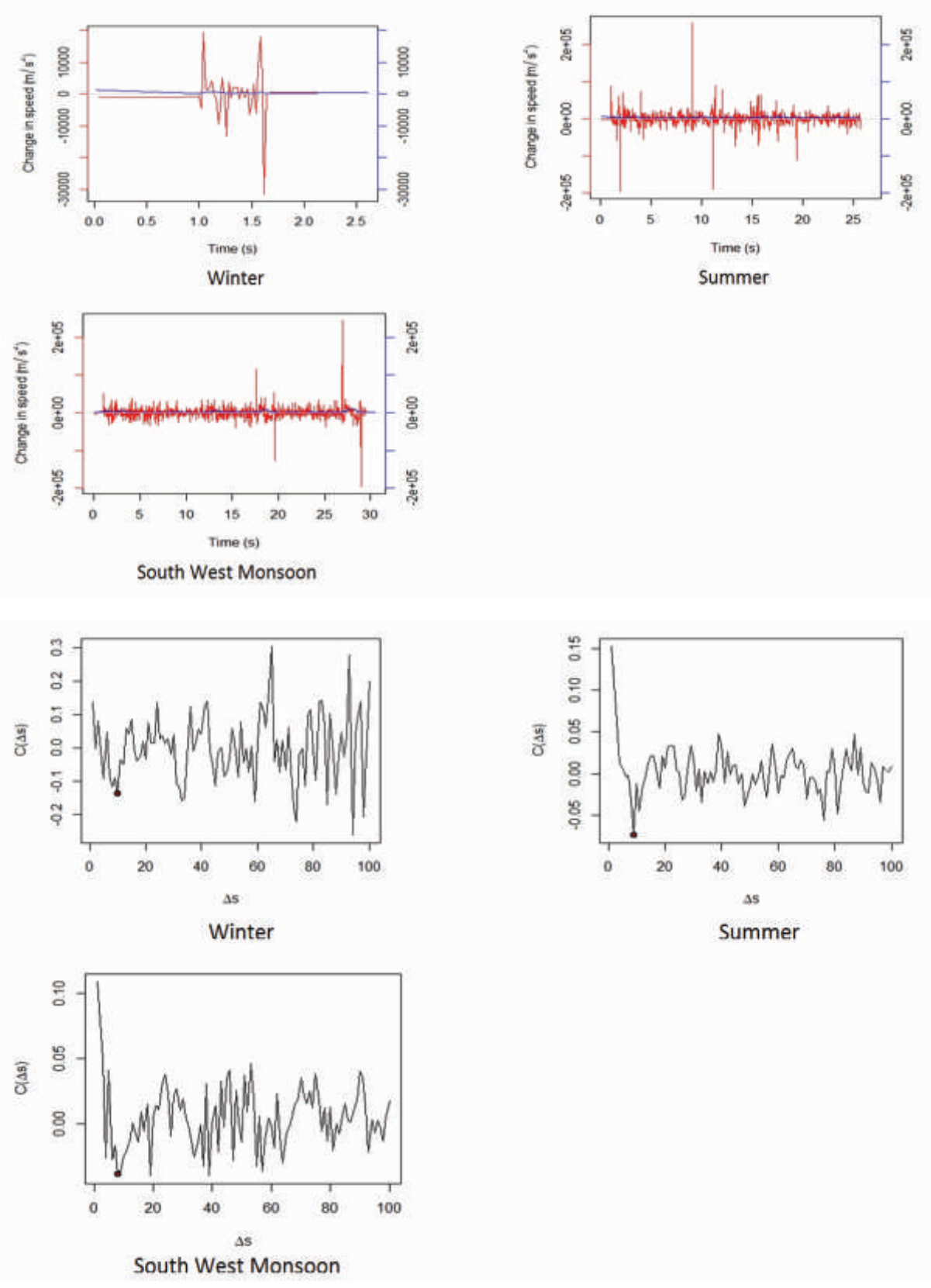

Figure 2.4 : *Acceleration, speed and Direction autocorrelation of trajectory of Aiyappa with respect to season*

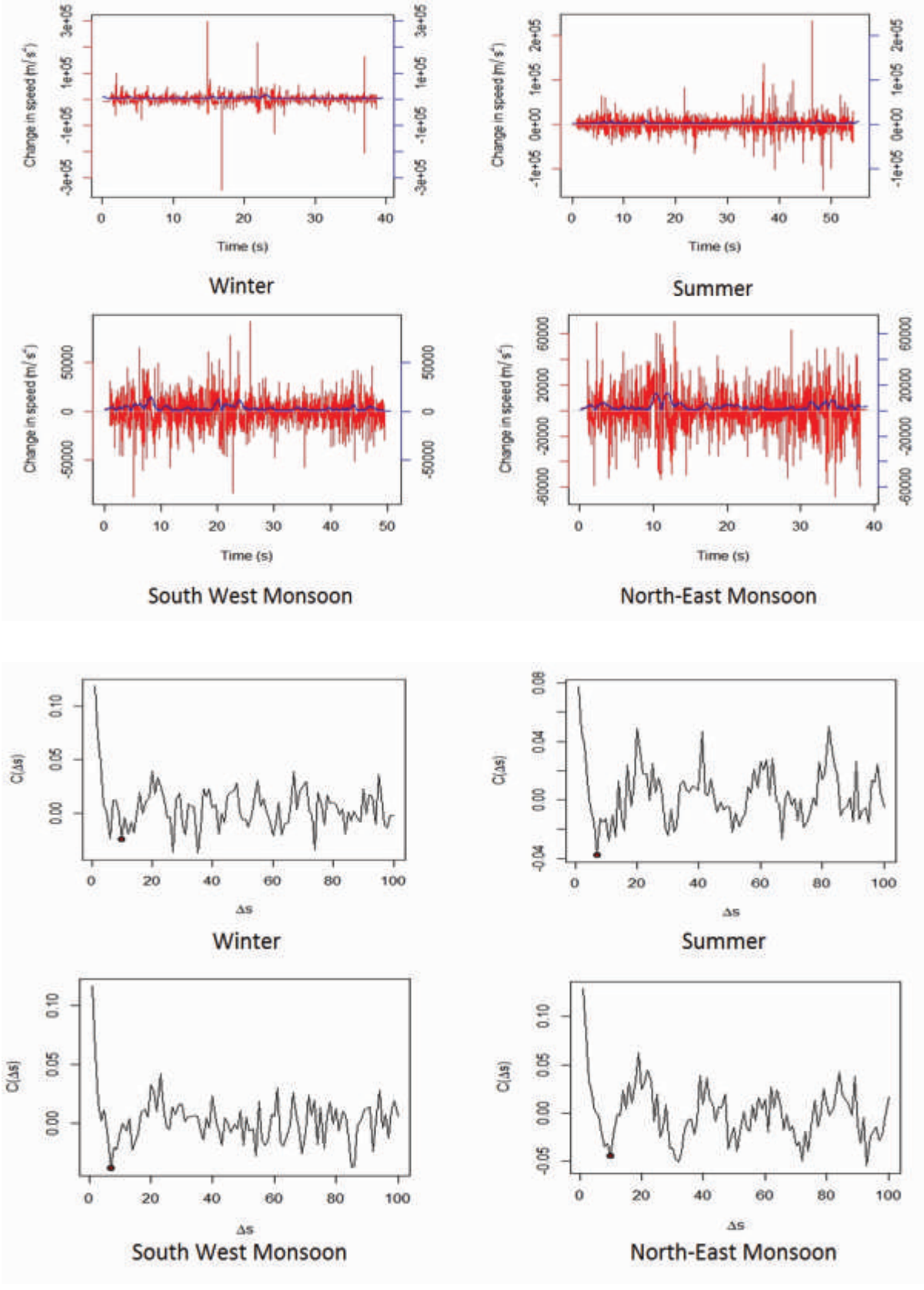

Figure 2.5 : *Acceleration, speed and Direction autocorrelation of trajectory of Beetamma with respect to season*

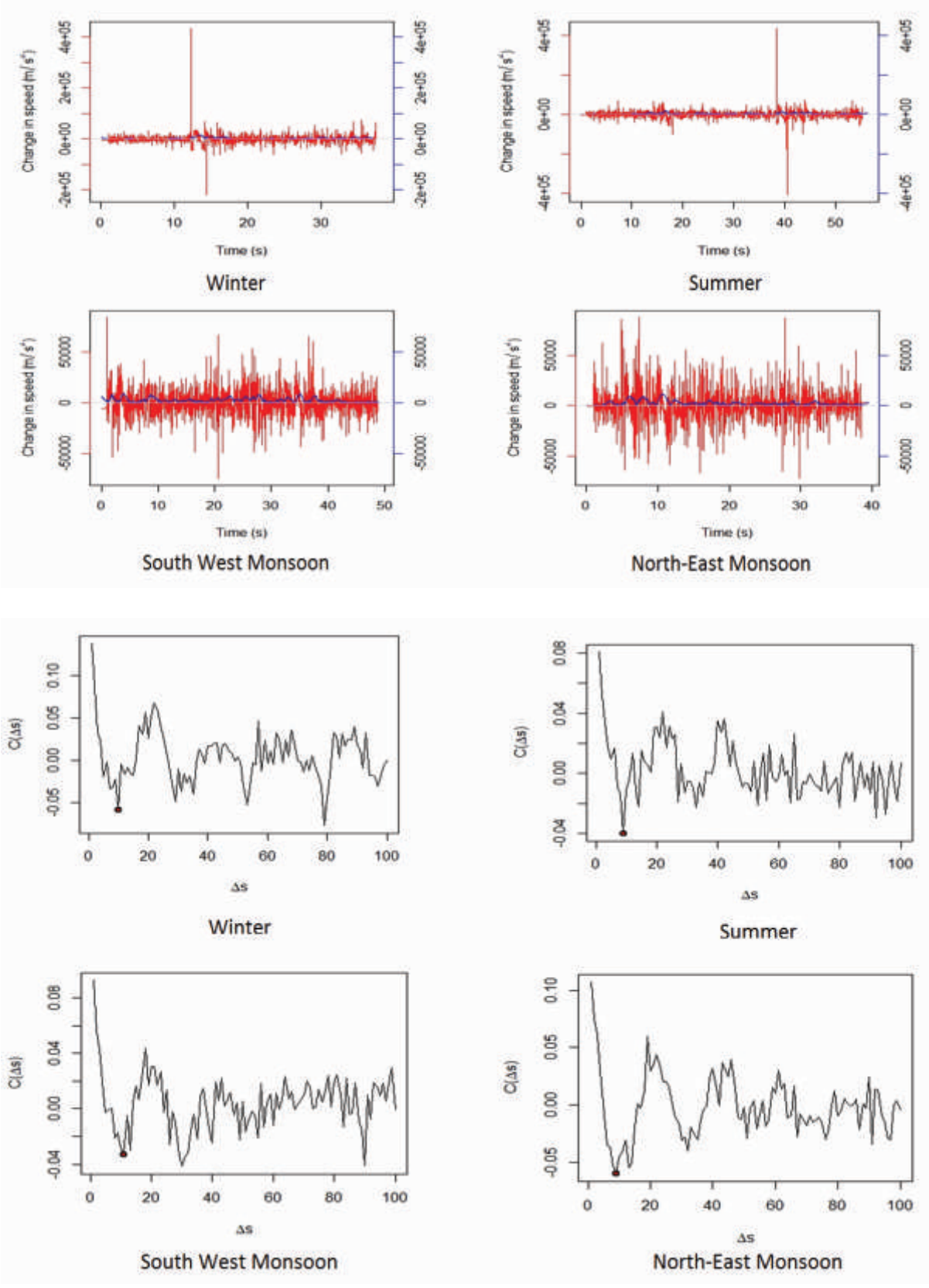

Figure 2.6 : *Acceleration, speed and Direction autocorrelation of trajectory of Bhuvneshwari with respect to season*

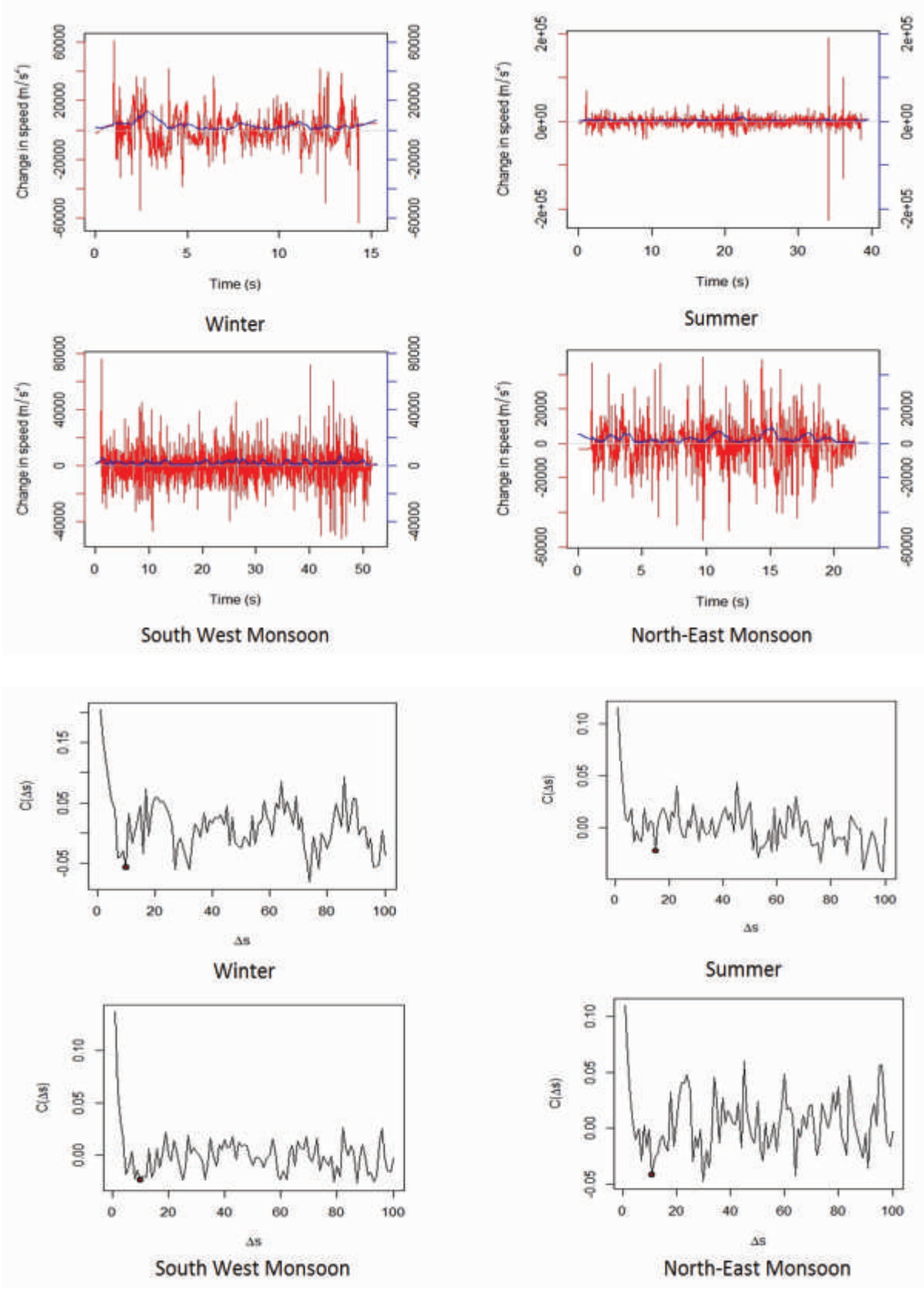

Figure 2.7 : *Acceleration, speed and Direction autocorrelation of trajectory of Oldbelt with respect to season*

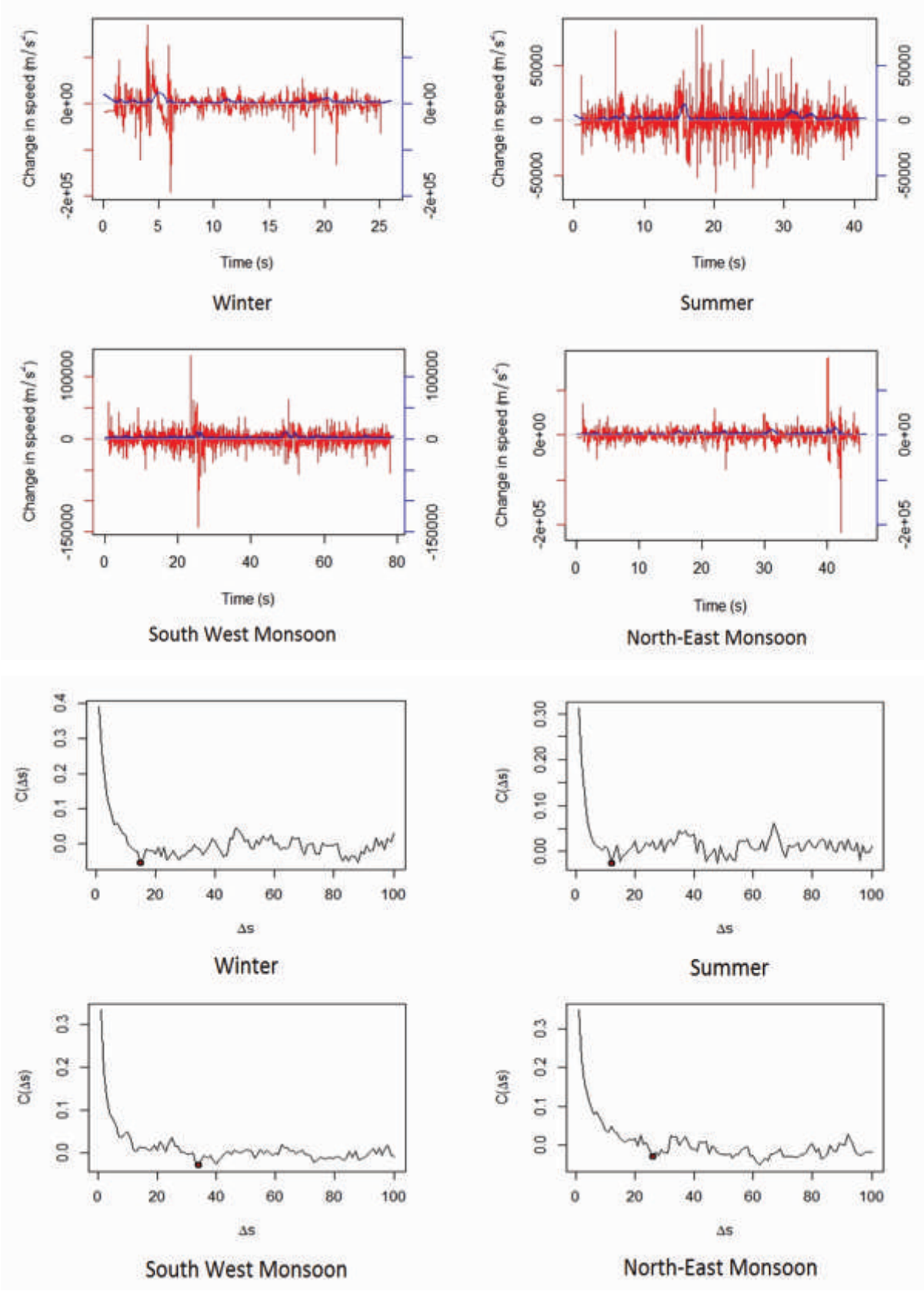

Figure 2.8 : *Acceleration, speed and Direction autocorrelation of trajectory of NE2 with respect to season*

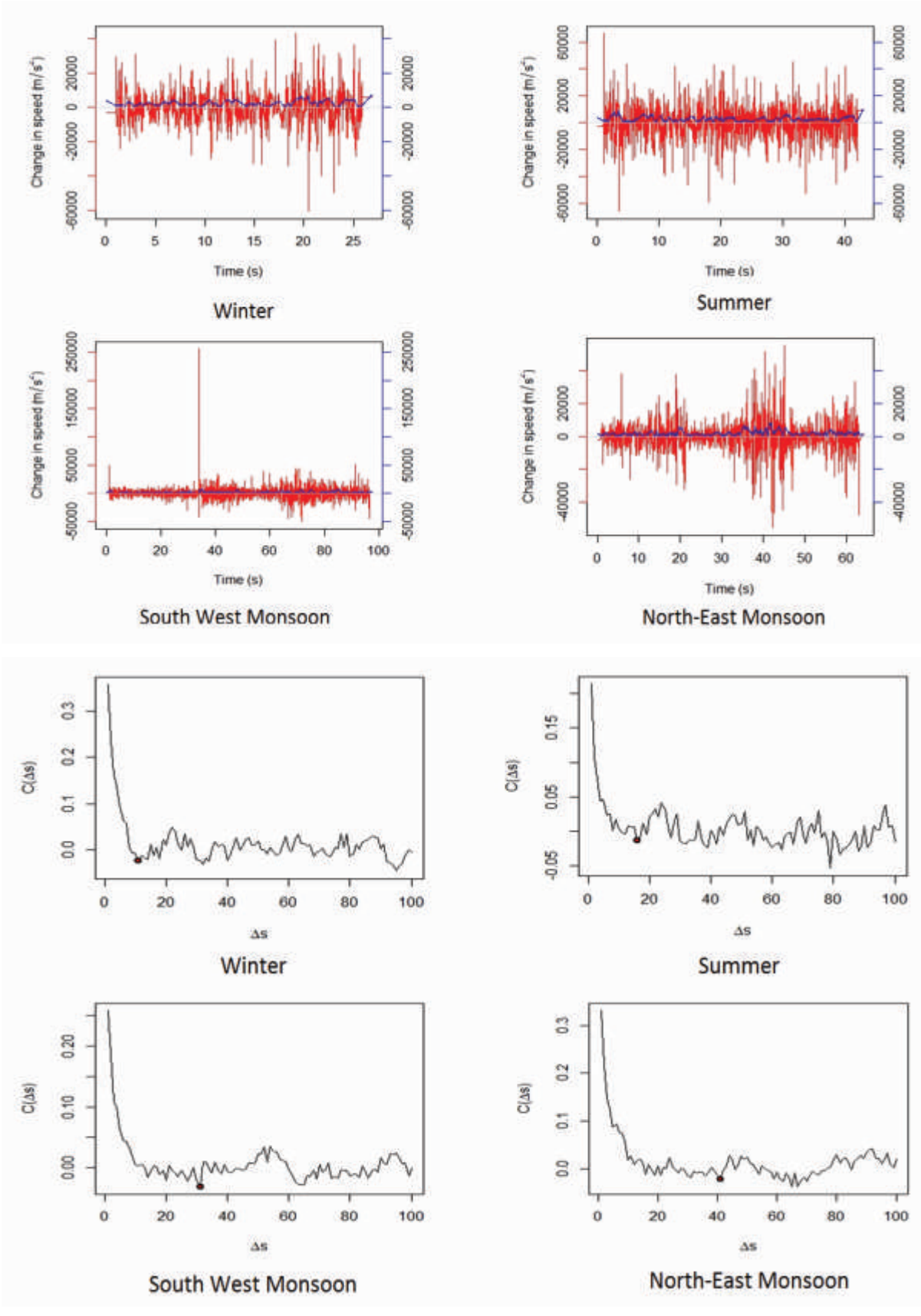

Figure 2.9 : *Acceleration, speed and Direction autocorrelation of trajectory of NE4 with respect to season*

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